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ERYTHEMA ARTHRITICUM EPIDEMICUM (HAVERHILL FEVER)

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In January 1926 a small but explosive outbreak of illness occurred in a restricted area of Haverhill, Mass. Several physicians called by patients in this epidemic were struck by its unusual features, especially the abruptness of the onset, the peculiar eruption and the marked involvement of the joints.

On January 22 we were called in consultation by the department of health. A preliminary report was made in February 1926.[1] A complete bacteriologic report was made by Parker and Hudson[2] in September 1926. The present paper is a complete report of the findings in this epidemic.

The only previous epidemic of a disease similar to this of which we have found a record occurred in May and June 1925, at Chester, Pa. Studies of this outbreak were made by Dr. Charles Armstrong[3] and Dr. Harold B. Wood,[4] state epidemiologist. In that epidemic about 400 cases were discovered in May and June 1925. It is estimated that about 600 cases occurred with no deaths. Although the nature of the epidemic was not determined, there was a striking similarity of onset, symptoms and course and epidemiologic relation to the milk supply to the disease reported here.

The onset was unusually abrupt, with severe chills, fever, vomiting, headache and pains in the back and joints. There was a remission of fever after a few days. The rash appeared at varying times in the early period of the disease as a macular or papular and often petechial eruption on the wrists, arms, feet and ankles. In a few cases it became pustular. The white blood cell count varied in 6 cases from 6,000 to 15,000. The joints were painful and tender, in some cases showing

From the South Department, Boston City Hospital.

1. Place, E. H.; Sutton, L. E., and Willner, O.: Erythema Arthriticum Epidemicum; Preliminary Report, Boston M. & S. J. **194**:285, 1926.

2. Parker, Frederick, Jr., and Hudson, N. Paul: The Etiology of Haverhill Fever (Erythema Arthriticum Epidemicum), Am. J. Path. **2**:357 (Sept.) 1926.

3. Armstrong, Charles: Milk-Borne Outbreak at Chester, Pa. Report rendered July 18, 1925; personal communication.

4. Wood, Harold B.: Personal communication to the authors.

no local signs, but in others swelling and occasionally redness. Pain and stiffness in the joints persisted after the subsidence of fever.

Although dengue was the working diagnosis during the early part of the epidemic, a study of the mosquitoes showed no unusual number and none at all of Aedes aegypti.

It was soon found that the milk supply was responsible. Ninety-two per cent of the known patients received raw milk from a bottling plant in the city. About 20 per cent of the persons receiving milk from this source had the disease as compared with 0.5 per cent of the population as a whole. In 250 homes in which the disease occurred there were 1,194 persons, 33 per cent of whom had the disease. Of the 8 per cent of patients not receiving milk from the bottling plant nearly one half had taken the infected milk in restaurants, leaving only 4.5 per cent of the known cases which were not definitely traceable to the milk. On one of the six farms supplying milk to the bottling plant was found the first person known to have had the disease. This person became ill four days before the first customer to contract the disease became ill. In the first six days after the onset of the first case 5 other cases appeared, including that of one of the proprietors of the bottling plant. Shortly afterward the outbreak became explosive.

From a few observations the period of incubation in the Chester epidemic was believed to be from two to four days.

Two blood cultures in the acute stage were negative. Forty-two per cent of throat cultures from 38 patients were positive for streptococci. Of 18 patients still more or less ill with sore throat, streptococci were found in 61 per cent, and in 31 per cent of 13 who had recovered. Of 7 persons who had not been ill, 14 per cent had positive streptococcus cultures. There was considerable variation in the hemolyzing power of the streptococci isolated.

A definite decision as to the nature of the disease was not made for opinions varied from dengue fever to streptococcic sore throat.

Levaditi, Nicolau and Poincloux [5] described an infection in one of their co-workers which clinically resembled this disease, and an organism, Streptobacillus moniliformis, which appears to have been the etiologic agent, was isolated from the blood during the acute stage. It has characteristics similar to those of the organism isolated in the Haverhill epidemic. Their report is of a single case occurring in March 1925 with recovery.

Earlier than that we have not found any report of a clinical entity with bacteriologic findings which resemble those in the Haverhill epidemic.

5. Levaditi, C.; Nicolau, S., and Poincloux, P.: On the Etiologic Rôle of the Streptobacillus Moniliformis in Acute Septicemic Polymorphic Erythema, Compt. rend. Acad. d. sc. **180**:1188, 1925; Research on the Etiology of an Acute Polymorphic Erythema, Presse méd. **34**:340 (March 17) 1926.

In 1932 Hazard and Goodkind [6] reported a case in which Haverhill fever was diagnosed in the wards of the Boston City Hospital, with recovery from the blood at three different times of an organism of the same characteristics as that isolated in the Haverhill epidemic.

Levaditi, Selbie and Schoen [7] recently reported the isolation of Streptobacillus moniliformis (Levaditi) from mice ill with spontaneous polyarthritis.

In a number of cases of fever following the bite of a rat organisms have been obtained from the blood similar to and possibly identical with Haverhillia multiformis by Schottmueller,[8] Blake,[9] Litterer,[10] Tunnicliff and Mayer,[11] Ebert and Hesse [12] and Mackie and McDermott,[13] although the latter later found a spirillum on inoculation of animals.

THE ETIOLOGIC ORGANISM IN THE HAVERHILL EPIDEMIC

In the Haverhill cases an organism was found by Parker and Hudson [2] in blood cultures taken by us in 11 of 17 cases and in fluid aspirated by us from the knee joints in 2 cases. They named the organism Haverhillia multiformis, thereby making a new genus Haverhillia in the family Mycobacteriaceae (Chester) in the order Actinomycetales (Buchanan). Serum from the patients produced agglutination of a polyvalent antigen of this organism in from 1 : 50 to 1 : 100 dilution, while serum from normal persons failed to show agglutination in dilutions higher than 1 : 20. Skin tests with the organism were positive in 15 of 18 cases from four to five months after the infection and negative in 10 controls.

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6. Hazard, J. B., and Goodkind, R.: Haverhill Fever; Report of a Case and Bacteriologic Study, J. A. M. A. **99**:534 (Aug. 13) 1932.

7. Levaditi, C.; Selbie, F. R., and Schoen, R.: Arthritis of Spontaneous Polyarticular Rheumatism of the Mouse Produced by Streptobacillus Moniliformis, Compt. rend. Soc. de biol. **103**:1193 (May) 1930. Levaditi, C., and Selbie, F. R.: Mode of Transmission of Acute Polymorphic Multiforme Erythema, Compt. rend. Acad. d. sc. **189**:1332, 1929.

8. Schottmueller, H.: Etiology and Clinical Characteristics of Rat-Bite Fever, Dermat. Wchnschr. (supp.) **58**:77, 1914.

9. Blake, F. G.: The Etiology of Rat-Bite Fever, J. Exper. Med. **23**:39 (Jan.) 1916.

10. Litterer, William A.: New Species of Streptothrix Isolated in a Case of Rat-Bite Fever, J. A. M. A. **68**:1287 (April 28) 1917.

11. Tunnicliff, Ruth, and Mayer, K.: A Case of Rat-Bite Fever, J. Infect. Dis. **23**:555 (Dec.) 1918.

12. Ebert, B., and Hesse, E.: On the Clinical Appearance and Bacteriology of the Japanese Rat-Bite Fever, Arch. f. klin. Chir. **136**:69, 1925.

13. Mackie, T. J., and McDermott, E. N.: Bacteriology and Observations in a Case of Rat-Bite Fever: Spirillum Minus, J. Path. & Bact. **29**:493 (Oct.) 1926.

Koch's postulates were not proved as there was no experimental inoculation of man, but it is felt that the evidence of the organism found in the blood stream and pathologic lesions, properly controlled, as well as the appearance of immune bodies in the infected patients is sufficient for regarding this bacterium as the etiologic agent.

EPIDEMIOLOGY

Haverhill is a manufacturing city. At the time of the epidemic it had a population of 35,000. On Jan. 22, 1926, twenty days after the appearance of the first case, the local board of health was notified by a local physician who had seen personally 8 cases that day. Up to that time there had been 71 cases. The following day the survey for the

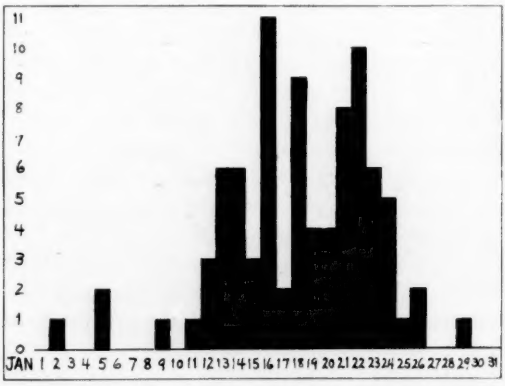

Fig. 1.—A graph showing the chronological occurrence of cases.

epidemic was begun. From then on 15 cases appeared before the epidemic was stopped. Three cases appeared during the first week, 31 during the second and 37 during the third. The epidemic reached its height at the end of the third week. Figure 1 shows the chronological incidence.

The epidemic was limited to a small district bordering the river, rectangular in shape, measuring about 5,000 by 1,500 feet (1,500 by 500 meters), including two roughly parallel streets and the short intersecting ones. Within this area 86 cases developed (fig. 2). The patients were chiefly of foreign extraction; Lithuanians, Italians, Poles and Jews (in the order named as to frequency) appeared to be the chief inhabitants of the infected district.

Thirty-nine families were involved, and 21 of these had more than 1 case. In a family of 18, including the grand-parents, parents and children, there were 10 cases, the largest group in any family.

The ages of the patients varied from 8 months to 54 years. No nursing babes were among those infected. The age classification was:

	Number	Per Cent
Under 2 years	7	8.2
2 to 12 years	31	36.0
12 to 21 years	17	19.8
21 to 54 years	31	36.0

Fifty-one, or 59 per cent, of the patients were females and 35, or 41 per cent, were males.

Almost all of the men were occupied in shoe factories and in the neighboring provision stores.

The living conditions of the homes were from poor to moderate, with the majority of the homes clean and neat. Every house had an inside toilet; there were no outside privies. The kitchen was the most

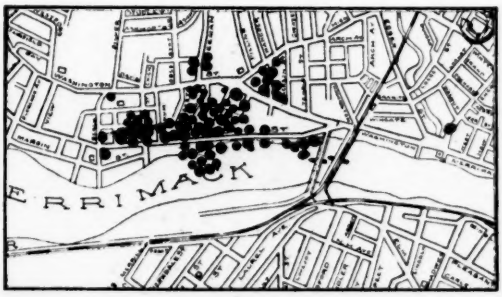

Fig. 2.—Map of Haverhill, Mass., showing the distribution of cases. Each case is represented by a dot.

used room in most of the homes, and in some homes in which the conditions were poor there were many sleeping in a room. As a whole most of the rooms showed plenty of light and sunshine. Rats were present in a few of the houses, but no vermin were found. Flies and mosquitoes were not present, as the epidemic appeared in January, and there was much snow.

Running water was supplied in every house from the city reservoir. The city health department reported that nothing had happened to the city's water supply recently to suggest that the water might be contaminated, and no cases had occurred in other parts of the town using the same water supply.

The food supply was obtained from the neighboring provision stores. The majority of the patients, except the infants, stated that they had eaten raw food lately, such as lettuce and occasionally celery. Raw

meat was eaten by 1 patient, but the rest claimed that their meat was always cooked. Fruits such as apples, oranges, bananas and pineapples were eaten by many.

The milk supply in every case came from one dairy either directly or through four stores in which this dairy's milk was sold. One patient, however, ate only ice cream made from this milk. The entire milk supply of this dairy was distributed to a small area of the city which corresponded to the area of the disease. The distributor daily supplied 18 quarts (19 liters) of milk directly to eleven residences and 62 quarts (65 liters) to four stores, which accounted for the dairy's entire supply.

Of the 39 families presenting cases of the disease 25 secured milk from stores supplied by this dairy alone, and 6 took milk from stores supplied by this as well as other dairies. The other 8 families had delivery of milk from the dairy in question direct to the home.

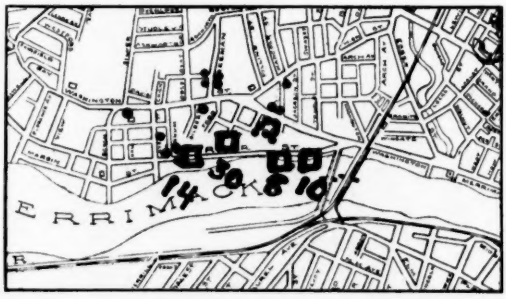

Fig. 3.—Map of Haverhill, Mass., showing the distribution of milk carrying the infection. Each dot represents a family taking milk directly from the dairy. The squares represent stores, with the number of quarts received by each in the adjacent figures.

Two hundred and thirty-one persons were included in the group, of whom 84, or 36 per cent, had the infection, an average of 2.1 cases per family. In several of the families some members rarely or never drank milk, and these usually escaped infection. In several only the persons who drank milk contracted the disease. Two cases occurred in families not receiving milk from the dairy, but both patients had partaken of the milk in homes having this supply. One woman using milk from the suspected dairy always boiled it, and she remained well.

The milk was ordered pasteurized on January 27, and after that only 1 case developed, two days later. The patient gave the history of having drunk the milk unpasteurized.

No cases developed through contact. Every patient gave a history of the ingestion of milk or its products. In some families there were

several sleeping in a room, but no case occurred through contact. None of the medical staff working in the field, hospitals or laboratory had the disease, even though it was impossible to carry out perfect aseptic technic.

In 17 cases in which there was some soreness of the throat, cultures were taken at varying times from the first to the twenty-seventh day of the disease. No unusual organisms were found. The usual flora of the mouth was present, including staphylococci, pneumococci, streptococci, etc.

The dairy was located several miles outside the city, and the milk was hauled to the customers every morning. Twelve cows were maintained, none of which was ill. One cow freshened from one to two months before the beginning of the epidemic, and her milk was added four or five days later. To make up a supply of 80 quarts (84 liters) two cans were received daily from two other dairies nearby. Examination of the herds by the city veterinarian gave negative results, and cultures from the milk proved negative. The water supply was from two wells.

The residents of the farm were the dairyman, his wife and 6 children, varying in age from 1 to 20 years. Within the past six months there had been no history of illness in the family, and an examination gave negative results. They stated that they used the milk. The sanitary condition of the dairy was as good as could be expected, but there was little modern equipment. Pasteurization was not done, as the saving of the expense of pasteurization allowed the milk to be sold 1 cent cheaper than that of the competitors. By the time the milk was pasteurized the epidemic had nearly ceased, as almost every customer who continually drank the milk had had the disease.

The portal of entry is thought to be intestinal. The only two epidemics reported have been shown to have been due to the ingestion of milk. The absence of cases through contact tends to exclude the respiratory tract as a portal of exit.

The disease appeared both in January and in June, making insect or seasonal factors apparently of little import.

During the epidemic in Haverhill we carried blood bouillon cultures by motor 35 miles (56 kilometers) to Boston in freezing weather without destroying the organisms. The blood did not freeze, however.

PERIOD OF INCUBATION

The time of ingestion of the infected milk in relation to the onset could not be told in most cases as the milk from the same dairy had been used continually. In 3 cases the partaking of the infected milk preceded the symptoms one, two and from two to three days, respectively.

In one of these cases a woman, 28 years old, who nursed in the household of a friend, took milk on January 21 and 22. Symptoms developed on the night of January 22. She took none of the infected supply elsewhere. In a second case a baby, 8 months old (who was at first believed not to have taken the milk), visited with her mother, and partook of the infected supply on January 21 and 22. She became ill on January 23. In the third, a man, 22 years old, came from a Boston hospital on the night of January 25 to nurse his father in the twelfth day of illness. He drank milk on January 26 and 27; the disease developed on January 29. This milk was pasteurized after January 27.

It is probable that the period of incubation varies with the amount of organisms ingested.

SOURCE

The source of infection is obscure. The causative organism had been previously noted only once, and that was not known to us at the time. It may have secured entrance to the milk from bovine sources, although its poor growth in milk makes the udder improbable as a focus. One cow showed antibodies in the blood, but routine examination revealed only a slight superficial lesion of one teat and dripping from another. On a visit to the dairy after agglutinins were discovered in her blood, it was found that this cow alone had been disposed of.

Contamination of the milk from water or handling through contaminated vessels is possible, as no sterilization was done, the simple equipment for that purpose being out of order at the time. Contamination from fodder such as ensilage has not been excluded.

The work of Levaditi and his co-workers and of Strangeways [14] showed that mice, and especially rats, are the natural carriers of Streptobacillus moniliformis. Levaditi, Selbie and Delorme [15] showed that the infection in mice with multiple arthritis or septicemia may be conveyed to other mice if kept together in the same jar in the dark, but not if kept in the light. They found the organism in the urine in the bladder, and believed that the infection is spread by the contamination of food in this manner as well as by bites. Strangeways found Streptobacillus moniliformis in the nasopharynx of 50 per cent of rats bred in the laboratory and in four wild rats, but not in the blood or organs. A study of the rats and mice at the dairy was not made, but the possibility of this source is intriguing.

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14. Strangeways, W. J.: Rats and Carriers of Streptobacillus Moniliformis, J. Path. & Bact. **37**:45 (July) 1933.

15. Levaditi, C.; Selbie, F. R., and Delorme, M.: Mode of Spontaneous Transmission of Infectious Polyarticular Rheumatism Caused by Streptobacillus Moniliformis, Compt. rend. Soc. de biol. **107**:501, 1931.

The identity of Streptobacillus moniliformis, Streptothrix muris-ratti and Haverhillia multiformis requires further study, but at present they show too little difference to suggest more than different strains. Levaditi found that Streptobacillus moniliformis of human source grew in milk, and that rabbits were the most susceptible animals, while the strain from mice was much more virulent for mice than for rabbits.

If the evidence of the short period of incubation can be accepted the milk supply must have been repeatedly or continually infected for a period of over three weeks.

CLINICAL PICTURE

Onset.—The onset in most cases was acute. Rarely, there was an indefinite history of malaise, of pain in the extremities and of headache gradually appearing in the course of a couple of days. Most gave the history of being perfectly well until suddenly a chill, vomiting or a severe headache ushered in the illness. In many cases the onset was extraordinarily abrupt, the patient being seized by a sudden violent chill while at work or asleep. In some cases a sudden and severe headache, vomiting, prostration and malaise interrupted their daily routine. Convulsions occurred in infants or very young children.

The fever tended to show a remission to normal in from two to five days and the symptoms were so ameliorated by the third or fourth day that many of the patients got out of bed, and several started back to work, to return to bed later with the recurrence of fever. The fever recurred two or three days after the remission to normal, the recurrence coinciding with the onset of generalized involvement of the joints. The eruption began usually during the primary fever, but tended to persist or even to increase into the relapsing period.

The chief symptoms of the 86 patients and their appearance during the first stage of the disease were:

	Number	Per Cent
Fever	83	96.5
Vomiting	53	61.6
Headache	48	55.8
Chills	47	54.7
Chilliness	11	12.8
Dizziness	14	16.2
Somnolence	7	8.1
Irritability	4	4.6
Herpes	4	4.6
Backache	4	4.6
Delirium	4	4.6
Epistaxis	3	3.5
Nausea	3	3.5
Convulsions	2	2.3
Anorexia	2	2.3
Photophobia	1	1.1
Tingling and numbness	1	1.1
Shooting pains	1	1.1

Fever.—Every patient examined during the acute stage showed fever, either slight or a temperature as high as 105 F. The 3 patients reported as free from fever were without records of temperature.

The typical temperature curve was a sudden rise following a chill or chilliness, its height being reached within twenty-four hours. A drop in temperature to normal occurred almost as rapidly, in from twenty-four to forty-eight hours in most cases. When chilliness persisted, the temperature remained up. The symptoms abated as the

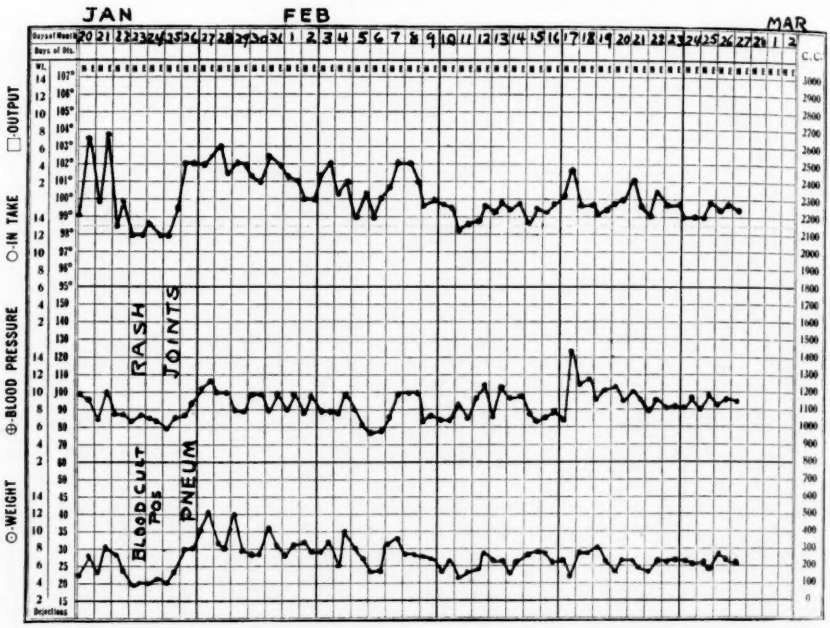

Fig. 4.—A characteristic temperature curve in a woman, aged 44, from the first day.

temperature fell. In some cases in which the stages merged the elevation of the temperature persisted at a lower level. As a rule when the rash appeared the temperature was either normal or nearly normal.

When the second stage set in the temperature was elevated and remained so for a few days or weeks, depending on the severity of the arthritis. Few of the patients showed no rise of temperature with a somewhat mild arthritis; others had a temperature as high as 103 F. and throughout several weeks averaging about 100 F. The majority of the fevers, however, were septic, the temperature gradually reaching normal in about a week.

Pulse and Respiratory Rates.—The pulse rate varied with the patient. It showed no marked relative increase or decrease, but varied with the temperature. The respiratory rate showed nothing abnormal. There was an increase in those patients who showed pneumonic conditions in relation to the amount of pathologic change.

Vomiting.—This occurred in 61 per cent of the cases. In a little over half of these vomiting occurred more than once, averaging three or four times. In the severer cases retching was present, with bile-

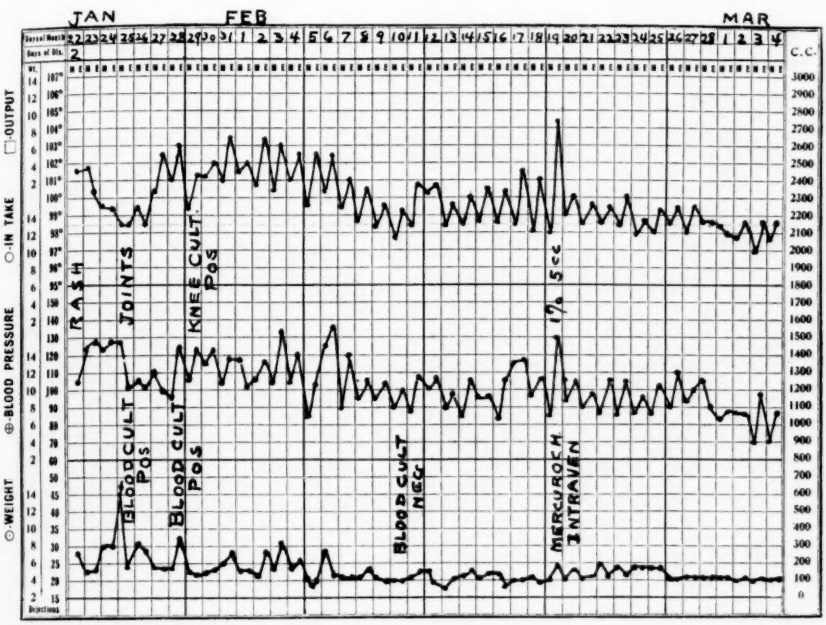

Fig. 5.—A characteristic temperature curve in a girl, aged 13, from the second day.

stained vomitus. One patient stated that he vomited "pure blood." Vomiting occurred over two days and from three to eight times in 7 per cent of the patients. One patient, a girl of 5 years, vomited thirty times over a period of three days. A small percentage (3½) of the patients did not vomit, but showed nausea.

Anorexia was present in 2 patients who did not show nausea or vomiting, and was more or less evident in all cases.

Chills.—Definite chills occurred in 55 per cent of the cases (2 patients stated that their teeth were almost shaken out), and, when present, generally were the first symptom. As a rule they lasted for

fifteen or twenty minutes, several patients claiming chills of a duration of one half hour. A few had repeated chills over a period of two days, and 1 for three days. Cyanosis was marked in 2 infants of the same family, aged 10 months and 2 years. The former had seven definite chills over a period of two days, and the latter had six during the same time. Thirteen per cent showed chilliness, but not marked enough to be classified as chills. The chilliness was of one, two or three days' duration, and in 1 case one week.

Convulsions were present in 2 children, aged 3 and 5, who did not have definite chills. In the former there was one convulsion which lasted for fifteen minutes; the other had two attacks of about the same duration.

Headache and Other Like Disturbances.—This occurred in 56 per cent of the cases, and was fairly severe. As a rule the headache was generalized, but when limited it was in the frontal region. The duration of the headache varied from an hour to a week, but in the majority of cases the duration was from twenty-four to forty-eight hours.

Dizziness was present in 16 per cent, occurring in the severer cases and lasting through the height of the toxic symptoms. Somnolence was complained of by 8 per cent, mostly adults. One patient, unable to sleep for three days, was afflicted the most. Restlessness and irritability were found in the infants and small children in a few cases. Severe backache was present in 4 patients. The rest showed more or less general malaise. The sicker patients showed prostration. Delirium occurred in 4 cases; in 2 uncomplicated cases at the onset; in another on the fifth day of the disease, and in the fourth in complicating pneumonia which developed five days after the onset.

Photophobia was complained of by 1 adult, who also had blurred vision for twenty-four hours. Tingling and numbness of the right hand occurred in 1 adult on the second day of the disease, and lasted for several days. Stabbing pain, like needles, in the elbows shooting down the arms to the wrists persisted for two days in another adult. Herpes simplex labialis was present in 4 patients.

Epistaxis.—This occurred in 3, but was of slight degree.

Rash.—The eruption appeared anywhere from the first to the fifth day of the disease. In 1 case the eruption appeared as late as the eighth day. In 7 cases the eruption appeared on the day of onset, in 16 on the second day, in 3 on the third, in 10 on the fourth, in 7 on the fifth and in 1, as previously mentioned, on the eighth day. In almost one-half the cases it appeared on the third day, when the temperature usually returned to normal.

It was most marked at the distal ends of the extremities, especially on the lateral or extensor surfaces and occasionally with accentuation

around the joints. Frequently it extended down the backs of the hands and the dorsa of the feet and less frequently over on the palmar and plantar surfaces. In the severer cases the exanthem was not limited to the extremities, but was present on the forehead and the sides of the face and on the trunk, usually the chest and the back. The flexor surfaces of the body were usually free except in marked eruptions, when there was extension from the extensor surfaces.

The eruption showed blotchy, irregular, generally rounded, maculopapular, dull red lesions varying greatly in shape and arrangement, measuring from 1 or 2 to 3 or 4 mm. in diameter. In a few cases the lesions were larger, particularly when present on the face. In 1 case a few lesions measured almost 1 cm. in diameter. The rash varied in

Fig. 6.—Photograph showing the subsiding eruption on the fifth day, eight days after the onset of the disease.

type, being chiefly rubelliform, but in some cases tending toward morbilliform. The lesions showed no tendency to clear in the centers; they faded on pressure and on the whole were discrete except for an occasional small area of conglomerate lesions around the joints.

The eruption increased in intensity for from one to three days, and lasted from one to fourteen days. The average duration was six days. In the mildest cases the eruption was present for one day. In 1 case it lasted for three weeks. In the persisting eruptions pigmentation generally resulted lasting from two to three weeks.

Petechial hemorrhages were present along the dorsa, the inner aspects and the plantar surfaces of the feet in the most toxic cases. A few patients showed some over the lower part of the leg and lower part of the arm which remained for some time after the erythema had faded.

A tourniquet applied to the upper part of the arm produced hemorrhages in the lesions of the lower part of the arm within five minutes after its application. Hemorrhages and pigmentation were present in about 21 per cent of cases.

Fine desquamation appeared in 20 per cent of the cases. It was found along the distal end of the lower part of the arm and leg, and in several cases was profuse on the hands and feet, including the fingers and toes. It was present after the fading of the eruption, but accompanying the pigmentation. Pigmentation, hemorrhage or desquamation occurred only after an erythema stage.

The exanthem was recognized in 80 of the 86 cases, or 93 per cent. Since the presence of rash in some instances was determined by the history, it is probable that some rashes may have been overlooked, judging from the mentality of some of the patients. An eruption was present in all cases that were observed personally from the beginning of the disease. The distribution of the eruption in 80 patients, or 93 per cent, was as follows:

	Patients	Per Cent
Trunk, face, arms and legs	9	....
Trunk, arms and legs	4	....
Trunk and arms	1	....
Face, arms and legs	26	....
Arms and legs	25	....
Face and arms	5	....
Arms	8	....
Legs	4	....
Totals		
Trunk	15	17.4
Face	40	46.5
Arms	76	88.4
Legs	65	75.6

Arthritis.—This was the most persistent symptom. Its onset was from the second to the thirteenth day of the disease, and usually marked the beginning of the recurring fever. In the average case involvement of the joints began on the fourth or the fifth day of the disease; in almost all cases it was multiple, and in over half there were more than six joints involved. There were many remissions and recurrences. In addition to definite involvement of the joints there was generalized pain in the arms and legs with no local evidence in the joints. Some, chiefly infants and children, showed no definite involvement of the joints. About 16 per cent had generalized pain, of whom three fourths were infants and children. The vertebral joints were possibly involved, as there was great pain in turning or moving the neck or back.

Objectively the joints varied from a normal appearance to well marked swelling and slight redness. In the knee joint in at least 6 cases fluid was demonstrated by a floating patella. The joints were

exceedingly painful, crippling the patients, keeping them either in bed or in the house, and dominating the relapsing stage of the disease. Table 1 shows the extent of involvement of the joints in 83 patients.

In the epidemic in Chester, Pa., 1 patient complained of pain on moving the eyes.

TABLE 1.—*Joint Involvements*

Joints	Pain	Swelling	Redness	Totals: Involvement	Totals: Swelling or Redness or Both
Right shoulder	30	2	..	32	2
Left shoulder	30	4	..	34	4
Right elbow	35	8	1	44	9
Left elbow	34	8	2	44	10
Right wrist	32	13	1	46	14
Left wrist	30	11	1	42	12
Right fingers	19	8	5	32	13
Left fingers	20	9	4	33	13
Right hip	15	..	..	15	..
Left hip	16	1	..	17	1
Right knee	31	11	1	43	12
Left knee	26	13	..	39	13
Right ankle	21	8	2	31	10
Left ankle	21	6	2	29	8
Right foot (including toes)	14	3	..	17	3
Left foot (including toes)	14	3	..	17	3
Vertebrae of the neck	9	0	..	9	..
Vertebrae of the back	4	..	..	4	..
Clavicles	..	1	..	1	1
Pubes	..	1	..	1	1

The wrists and elbows were the joints most frequently involved, with the knees, shoulders, fingers and ankles following in order. The following tabulation shows the number of joints involved in each patient.

Joints Involved	Patients	Joints Involved	Patients
1	8	9	1
2	5	10	4
3	6	11	3
4	3	12	5
5	4	13	4
6	6	14	4
7	6	15	2
8	5	16	2

The 15 patients with generalized pain in the arms and legs are not included in the preceding list.

The shortest duration of involvement of the joints was two days, the longest several months. The involvement subsided within one week in 10 patients; within from one to two weeks in 12, and within from two to four weeks in 12. In 34 the joints were still involved at the end of a month, and in 3 at the end of five months. In the 34 cases were included those in which some definite pain or pain and swelling showed in any joint which had been involved from the onset. In the period of from one to five months the pain and swelling in most of the 34 cases subsided.

Paracentesis of two joints with fluid revealed yellow cloudy fluid in which on standing a slight flocculent precipitate appeared. Microscopic examination revealed polymorphonuclear leukocytes. Cultures taken from the fluids showed a pure culture of Haverhillia multiformis.

Fig. 7.—Photograph showing swelling of the joint of the left knee.

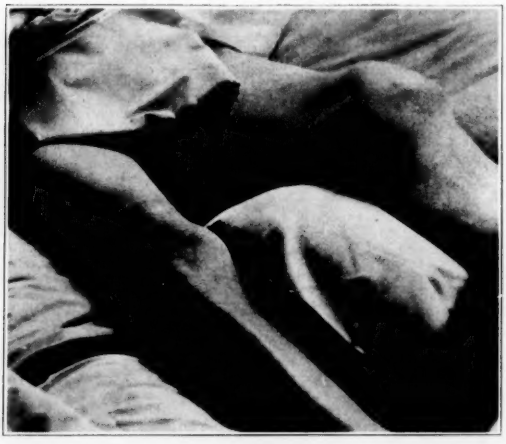

Fig. 8.—Photograph showing swelling of the joint of the left knee.

Respiratory Involvement.—Sore throat appeared in 39 cases, or a little less than one half. It appeared at any time from the first to the twenty-fifth day of the disease, and at the eighth or ninth day on the average. It came on most frequently during the stage of relapse. The duration was from one to twenty-five days, averaging six and a half days. The soreness was slight, dysphagia being present in only 3 cases.

The throat showed a diffuse dull redness with capillary injection extending over the soft palate. Cultures showed the organisms usually found in the throat. The tongue was slightly coated.

Cough occurred in 21 cases, and was more or less dry and nonproductive and not severe. It occurred with the slight sore throats. Two patients, aged 3, had croupy coughs during the onset of disease. Three patients complained of pain over the larynx with slight hoarseness, and slight tenderness on pressure over the larynx was present during the latter stage of the disease. One of these suffered loss of voice. Hoarseness in another lasted two weeks.

Bronchopneumonia developed in 2 cases on the third and fifth day of the disease and lasted about one week. One of the patients was markedly delirious, attempting to throw himself out of the window and requiring restraint. The physical signs were definite in both cases. Acute bronchitis occurred in 3 others, with cough and the raising of sputum without definite signs of pneumonia.

Organic Findings.—Apparently the heart was not affected by the disease. No cardiac enlargement was present. In only 4 patients were murmurs found, 3 caused by functional disorder and in the other due to an old arrested endocarditis with no signs of an acute flare-up. The pulse rate was relatively low.

The edge of the liver was palpable in 1 case, but was not tender, and no jaundice was present.

The edge of the spleen was palpable in 7 cases, but this was thought to be a coincidence.

Abdominal pain and distention were present in the 2 patients with pneumonia, while abdominal pain alone was found in several others during the onset, with vomiting, but lasted only a day.

The genito-urinary tract showed no symptoms, and appeared free from the infection. One patient showed frequency, which was thought to be a reflex action to the pain and swelling of the symphysis pubis, which was involved in the disease. This patient had normal urine. Urinalysis in 14 cases gave essentially negative results.

There was no enlargement of lymph nodes.

Blood.—The blood was examined in 23 patients from the first day to the fourth week of the disease. The composite picture of the white cell elements was: 11,500 white blood cells per cubic millimeter; 70 per cent ploymorphonuclears; 21 per cent lymphocytes; 6 per cent large monocytes; 2 per cent eosinophils, and 1 per cent basophils.

At the onset and throughout the disease the number of cells was just as often above the mean as below. The same can be said of the differential count. The white blood cell count varied from 5,680 to 18,888 (in a girl of 8 months). The polymorphonuclear count varied from 62 to 86 per cent. In babies and children the lymphocyte count

was higher, varying from 9 to 32 per cent. The large mononuclears varied from 4 to 11 per cent; the eosinophils from none to 6 per cent and the basophils from none to 2 per cent.

The red blood cell count varied from 3,728,000 to 4,456,000, the average being about 4,000,000. The hemoglobin ranged from 65 per cent, in a girl of 12 in the third week of the disease, to 95 per cent in a man in about the same stage of the disease. The average hemoglobin value was 80 per cent. The red cells showed some achromia, and the platelets in smears were normal.

TABLE 2.—*Blood Cultures of 17 Patients*

Patient	Date of Onset	Date of Culture	Day of Disease	Haverhillia Multiformis
A. A.	Jan. 21	Jan. 29	9	+
		Feb. 18	29	—
B. E.	Jan. 20	Jan. 23	4	+
		Feb. 23	35	—
B. L.	Jan. 21	Feb. 6	17	+
K. J.	Jan. 13	Jan. 23	11	+
		Feb. 6	25	+
K. M.	Jan. 29	Jan. 29	1	+
		Feb. 6	9	—
P. R.	Jan. 21	Jan. 25	5	+
		Jan. 28	8	+
		Feb. 10	21	—
H. W.	Jan. 19	Feb. 10	23	—
L. M.	Jan. 24	Jan. 24	1	+ (Contaminated)
L. E.	Jan. 20	Jan. 24	5	+
L. A.	Jan. 17	Feb. 20	35	Overheated
S. J.	Jan. 15	Feb. 6	23	+
R. A.	Jan. 23	Jan. 24	2	+
		Feb. 20	29	Overheated
P. L.	Jan. 18	Feb. 6	20	—
W. S.	Jan. 20	Jan. 24	5	+
C. G.	Jan. 21	Feb. 19	30	— (Contaminated)
G. M.	Jan. 21	Feb. 1	12	—
A. L.	Jan. 22	Feb. 1	11	— (Contaminated)

The blood pressures were essentially normal.

Blood cultures were taken in 17 cases, with the results shown in table 2.

The blood cultures were positive in 11 of 17 cases. There were positive cultures on the first day of the disease and in 1 case as late as the twenty-fifth day. The earliest negative culture was obtained on the ninth day of the disease. The earliest positive culture that was followed by a negative one later was from blood which yielded a growth of the organism on the first day of the disease and which gave no growth on the ninth. Repeated blood cultures were done only in 6 cases. Dark-field illumination of the blood in 2 cases showed no Haverhillia multiformis.

Involved Joints.—Cultures from taps of the joints on the ninth to eleventh days of the disease in 2 patients were positive. At this time both joints showed floating patellae. The cultures were not repeated.

Roentgen examination of an infected joint on the twenty-first day of the disease revealed the following: The left knee showed marked swelling of the soft tissue. The patella was pushed forward from the femur. The posterior part of the cartilage on the upper articular surface of the tibia had a fuzzy indistinct appearance. There was also a moth-eaten appearance of the border of the external condyle of the left femur.

Roentgen examination repeated on the thirty-third day of the disease revealed a degree of destruction over the external condyle of the left knee, which seems to have increased since the previous reading. Roentgen examination of a joint of another patient on the thirty-first day revealed a normal condition.

Spinal Fluid.—A lumbar puncture performed on the nineteenth day of the disease showed 25 cc. of clear fluid with some microscopic blood.

Relapse.—One case of the 86 showed a relapse.

Mrs. D., aged 33, on January 5 vomited four or five times, and had headache, backache and pain in the stomach. On January 6 she vomited four or five times. A rubelliaform exanthem appeared over the extensor surfaces of the arms. On January 7, the arms were painful on motion and to the touch. She could not raise her hands to her face or shoulders. The rash was still present. On January 8 swelling appeared with fluctuation of the left elbow; the rash was gone. On January 9 the pain in the hands, wrists, shoulder and right elbow was gone. On January 12 there was no pain in the left elbow, and the patient was well. Nine days later there was a relapse. On January 21 she vomited twice and had pain in the elbows and knees. On January 22 she vomited once, and there were pain in the left elbow and stabbing pain in both elbows shooting down her arms to her wrists. An exanthem appeared on the extensor surfaces of the lower parts of the arms and legs and feet. On January 24 the right knee was swollen and tender.

Levaditi and his co-workers reported relapses on the sixth and tenth days of the disease, each relapse being ushered in with an elevation of temperature, an appearance of a second and third eruption similar to the first and increased pain in the joints. Blood cultures were positive on the first two exacerbations.

Complications and Sequelae.—The complications were few. The pneumonia and bronchitis that accompanied the disease in some cases can be classified as such, but have been described under "Respiratory Involvement." In one case, two days before the pneumonia appeared, a positive blood culture was obtained. Whether the pneumonia was due to Haverhillia multiformis or to a secondary invader was not determined, as no puncture of the lung was done. The pneumonia was bronchial. One case showed involvement of the middle ear with no specific organ-

ism discovered. Toward the end of the course in 1 patient a typical streptococcic sore throat with membrane developed. The other mild sore throats are described under "Respiratory Involvement."

The skin over the extremities showed desquamation, and in 2 cases blebs appeared around the ankles. The organisms found were gram-positive diplococci and diphtheroids.

Of the patients in whom arthritis persisted for over five months, 2 still had recurring pain and swelling in the previously involved joints, associated with inclement weather, in September 1928. No changes in bone or cartilage were noted. One of the patients seen in September 1928 stated that he used crutches for seven months. Examination revealed a normal joint, but he stated that five days previously with the onset of rainy weather, the joint became swollen and painful. The discomfort lasted for three days, clearing with the weather. A second patient reported similar trouble with his joints. He is at present in a hospital for patients with tuberculosis. A third patient had moved away from the vicinity, and was not traced. Whether the etiologic agent still persists in these joints is doubtful. It is known that the blood of 2 of these patients was normal the last time blood cultures were made. It is possible that secondary causes are maintaining the arthritis.

PATHOLOGY

Essentially nothing is known about the pathologic processes, since in both epidemics there were no deaths and biopsy specimens were not made.

BACTERIOLOGY

Parker and Hudson [2] found in cultures from the blood and from the fluid of involved joints an organism, Haverhillia multiformis, of variable form, appearing chiefly as rods from 2 to 15 mm. long and from 0.2 to 0.5 mm. wide, sometimes paired, often as filaments or threads with true branching. A marked characteristic is the occurrence of a swelling or a knob at any point in the rod, from four to five times the diameter of the rod. This does not take the spore stain. The organism does not stain well with ordinary aniline dyes, is gram-negative and nonacid-fast.

Haverhillia grows best at atmospheric oxygen pressure or under a partially anaerobic condition in ascitic fluid broth or on solid medium composed of equal parts of glycerin extract of potato and infusion broth to which egg yolk is added.

The organism was most virulent for white mice, which it killed in from sixteen to forty-eight hours after intraperitoneal injection. Mice could be immunized by sublethal doses of living cultures or by dead cultures against fatal doses for control mice, this immunity persisting

for at least two months. The serum of immunized rabbits showed precipitins and agglutinins for the organism in dilutions up to 1 : 5,000, against all strains. The absorption of antibodies by one strain was complete.

IMMUNITY

Little evidence is available for conclusions as to natural or acquired immunity.

There were 231 persons in the families, in which the disease was recognized in 86 (37 per cent). A considerable number of these, however, partook of milk rarely or not at all. Fifty-one insisted that they took no milk during January, and the majority gave a history of small and infrequent partaking.

Eleven families received milk directly from the dairy. Of these, all but 1 had the disease, the exception being a family in which boiling the milk was practiced. Twenty-six infected families received the same supply through stores, but a reliable record of all who obtained the milk from the stores could not be obtained. Four persons became residents of infected households during the epidemic. The disease developed in 2 of these, and the other 2 took no milk.

The study of agglutinins in the blood of patients after recovery and in that of the uninvolved members of the families was not undertaken, as continued cooperation in getting specimens of blood, after the serious upsets the patients had gone through and with the complete removal of further danger, could not be secured.

Eighteen patients were tested intradermally with a killed culture of the organism on May 27, 1926, from one hundred and twenty-four to one hundred and forty-two days after the onset. Fifteen showed reactions from 1 to 3 cm. in diameter. Control tests with the medium were negative. Control tests on 10 persons who had not had the disease or taken the contaminated milk were negative. No tests were made in persons in the infected families who escaped the disease.

DIAGNOSIS

In epidemics it is doubtful if any difficulty in diagnosis would occur, even if blood cultures were not taken. The picture is characterized by: (1) abrupt onset with chills, fever, malaise, vomiting and headache; (2) an early eruption, rubellaform or morbilliform, occurring first, and often only, on the extremities especially about the ankles and wrists and tending to become hemorrhagic; (3) a multiple arthritis of varying, but often of a severe and crippling, degree, and (4) a fever curve of abrupt rise with remission in from two to five days, and after a few days of relative freedom from symptoms a recurrence with which the arthritic manifestations appear. In isolated cases, especially if

atypical, probably great clinical difficulties might occur. The frequent association of eruptions with manifestations in the joints in a number of distinct as well as obscure conditions makes the demonstration of the organism by blood culture or puncture of the joints essential to recognition.

Differential Diagnosis.—The common contagious eruptive diseases, as measles, rubella and scarlet fever, may readily be differentiated by the clinical pictures.

Rheumatic arthritis offers no difficulty, since the peculiar remittent (saddleback) fever of epidemic arthritic erythema has no counterpart in rheumatic fever. There is no tendency to cardiac involvement, and although manifestations in the throat are common, they tend to a diffuse catarrhal type of injection without exudate or tendency to involve the lymphoid tissue.

There are many points of similarity with dengue. The cause of the Chester epidemic was generally considered to be dengue. The abrupt onset with chills, fever, headache, malaise and generalized pains, the remission of fever after three or four days with a recurrence after two or three days of normal temperature, and the morbilliform rash on hands and feet, forearms and lower part of the legs are common to both. Postorbital tenderness or pain on moving the eyeballs is not evidenced in Haverhill fever. There is no tendency to suffusion of the eyes or congestion of the face, and no leukopenia with an increased percentage of lymphocytes. Hematemesis and jaundice are practically absent. The pulse rate is not especially low. Moreover, there is definite arthritis in most cases of Haverhill fever, although in many, objective signs may be slight and rarely absent. The rash, while similar, does not tend so commonly to involve the trunk extensively, and usually appears in the first febrile period instead of with the recurring fever as in dengue. The duration of the secondary fever and the arthritic symptoms is much more prolonged than in dengue and almost always lasts more than the two or three days noted in dengue.

Blood cultures would serve for positive differentiation of Haverhill fever.

During epidemics the epidemiology would serve, dengue being spread only by mosquitoes of the species Aedes aegypti (Stegomyia calopus), while Haverhill fever is spread only (?) by food.

Erythema infectiosum (Escherich), although accompanied by pain in the joints, differs in that the eruption appears on the sides of the face and is absent from the palms and soles, as well as by the development of larger circinate and serpiginous and coalescent lesions. The duration of Haverhill fever is usually much greater than the fever in infectious erythema (from five to ten days). Febrile reactions such as occurred in many of our cases are unknown in infectious erythema.

The eruption in erythema multiforme may at times simulate that seen in Haverhill fever. However, the larger, coalescent areas and the circinate, serpiginous and gyrate forms so characteristic of multiforme erythema were never seen in the cases of Haverhill fever. Involvement in the joints in erythema multiforme is certainly less likely to show definite swelling or to be so persistent as that in epidemic arthritic erythema. It is probable that some of the conditions classed in the large and somewhat obscure group of multiforme erythema, as shown by the report of Levaditi, Nicolau and Poincloux, are really identical with Haverhill fever.

Erythema subitum may be excluded by the appearance of the eruption in Haverhill fever frequently before the defervescence of the initial fever, the recurrence of fever, the arthritis and the absence of mononucleosis.

Influenza was a common diagnosis at the onset. The absence of cough and the widespread catarrhal signs of influenza, the appearance of the eruption, the recurrent fever with arthritis and the absence of leukopenia distinguish Haverhill fever.

Malta fever gives rise to some confusion, especially the form due to Brucella abortus. Both diseases are spread by contaminated milk and are characterized by tenderness and swelling of the joints, as well as by somewhat similar relapsing fevers. The more abrupt onset in Haverhill fever, the shorter initial fever as well as the relatively much shorter secondary fever would probably differentiate them. The involvement in the joints in Haverhill fever is often persistent, and while multiple does not tend to "skip about." There is no characteristic leukopenia with relative lymphocytosis and no splenomegalia. Neuritis and orchitis, so common in Malta fever, are absent in Haverhill fever. Blood cultures, agglutination tests with Brucella melitensis, Br. abortus and Br. paramelitensis and Burnet's intradermal test would certainly settle any doubt.

Rat-bite fever has an interesting but obscure relationship to Haverhill fever. It is probable that rat-bite fever is due to Spirochaeta morsus-muris (Futaki,[16] Kaneko and Okuda,[17] etc.), which has been found in the local lesions and the blood. Mooser [18] found this organism in the blood of a wild rat, Mus decumanus, and in the conjunctiva of rats and guinea-pigs experimentally inoculated through bites of ani-

16. Futaki, K.; Takaki, I.; Tamguchi, T., and Osumi, S.: Spirochaeta Morsus Muris, N. S. P., the Cause of Rat-Bite Fever, J. Exper. Med. **25**:33, 1917.

17. Kaneko, R., and Okuda, K.: Contribution to the Etiology and Pathology of Rat-Bite Fever, J. Exper. Med. **26**:363, 1917.

18. Mooser, H.: Spiral Organism Found in Wild Rat, J. Exper. Med. **39**:589, 1924.

mals suffering from the conjunctival and corneal infection. Whether the cases from which Streptothrix muris-ratti was isolated (Schottmueller,[8] Blake,[9] Tunnicliff[11]) represented a different variety of infection or were simply complicated by this organism remains uncertain. Strangeways[14] recently showed that Streptobacillus moniliformis is frequently present in the nasopharynx of laboratory rats and wild rats but not in the blood.

The reported cases of rat-bite fever in which Streptothrix muris-ratti has been found do not differ essentially from those showing Spirochaeta morsus-muris. The chief characteristics are: a variable incubation of from one or two days to eight weeks (Crohn[19]), but usually over two weeks; local cellulitis, lymphangitis and lymphadenitis; leukocytosis; an eruption, usually general but at times only in the region of the local inflammation and often from 2 to 3 cm. in diameter, more commonly appearing with the recurrent fever; chilliness; headache; pains in the muscles; tenderness of the nerve trunks, and a strikingly recurrent fever. The febrile period usually lasts from three to five days, may be single but usually recurs from three to ten times and occasionally many more over a period of days, weeks or months. The symptoms abate during the afebrile periods and show exacerbations, including the eruption, with the recurrent fever. No arthritis has been reported.

How much difference entry of the infection through a wound would make is not now evident, but Haverhill fever differs from rat-bite fever in the following characteristics: the incubation period is short (so far as known); leukocytosis is slight or absent; multiple recurrences of fever comparable to those in typical rat-bite fever are not found; the rash is smaller and more uniform in size, occurs especially on the extremities and does not recur with the return of fever, and the involvement of the joints is one of the characteristic features. Of course, no local inflammation with lymphatic involvement is present. The mortality of Haverhill fever is nil to date as compared with about 10 per cent for rat-bite fever. In Levaditi's case the eruption recurred with each febrile outbreak.[5]

PROGNOSIS

The prognosis is good. In the two epidemics of about 500 cases no deaths occurred. As to permanent crippling owing to involvement of the joints, it is difficult to say. Certainly few patients are left with residual effects. At the end of two and a half years only 3 of the 86 patients had symptoms: men, 54, 45 and 44 years of age. The first of these showed some contractures of the muscles of the thigh, although

19. Crohn, B. B.: Rat-Bite Fever, Arch. Int. Med. **15**:1014 (June) 1915.

the swelling in the knee had subsided entirely. On the eighty-fifth day of illness the x-ray picture showed a considerable degree of atrophy of bones and narrowing of the articular space—but no other positive change about the joint. An earlier report on the thirty-third day of the disease stated that there was a degree of destruction over the external condyle of the femur. The patient was discharged from bed. At his age, although all other joints have cleared, there appears to be little chance of his ever being fully well again. Of the other 2 men, 1 recently examined during the absence of swelling or pain showed no deformity or limitation of motion of any of the joints.

The arthritis of the children and young adults has all cleared. It can be concluded that early age favors the prognosis, for only 29 per cent of the patients under the age of 21 showed involvement of the joints at the end of eight weeks in comparison with 58 per cent of those over 21, a ratio of 1:2. Whether the joints that have been infected and healed are more susceptible to further involvement in later life is to be determined through the passing of time.

PROPHYLAXIS AND TREATMENT

Pasteurization of milk supplies should be required. Whether the disease can be spread by other means is not known, but other raw foods obviously might be factors. No contagion was evident.

No specific treatment was found. The patients had been treated expectantly with salicylates in various forms and hypnotics. Narcotics were freely used.

Our observations were begun three weeks after the outbreak of the disease when relatively few cases were available for therapeutic study. Sodium salicylate, acetylsalicylic acid and strontium were administered to the point of toxicity without relief. Quinine and the iodides also gave no definite result. To 1 patient still acutely sick in the third week 0.59 Gm. of neoarsphenamine was given intravenously. The temperature dropped to 96 F., but rose in four hours to 100 F. The patient was slightly better the next day. There was no change in the arthritis, which persisted in his case for months. One patient in the fourth week received 5 cc. of a 1 per cent mercurochrome solution intravenously; an hour later there occurred a severe chill lasting for thirty-five minutes followed by vomiting. The temperature rose to 104.2 F., to return within four hours to the previous level. There was no improvement in the condition in the joints or in the temperature curve.

Immobilization of the infected joints was usually done with supports and pillows. Splints were not usually used partly owing to the limitations of work in the field.

Decubitus had to be carefully guarded against. Diathermy and radiation treatments were used in some of the hospital cases by Dr. McFee with some symptomatic relief but little apparent effect on the course of the arthritis. Some contracture developed in the worst case, but great difficulty was found in managing the patient. The usual treatment of rest, high intake of fluid, oral hygiene, simple antiseptic sprays for the throat and occasionally oily sprays was followed.

SUMMARY

An epidemic of a disease, apparently a new clinical entity, named epidemic arthritic erythema or Haverhill fever is described. It is characterized by an abrupt onset, often with a chill, a rubellaform to morbilliform eruption, often scanty, chiefly on the extremities, with a tendency toward hemorrhage into the lesions, and an inflammation of the joints with marked pain and tenderness not infrequently of prolonged duration.

The disease occurred as a markedly localized epidemic and was undoubtedly spread through the raw milk supply.

An organism, described only once before, is believed to be the cause, having been found in the blood stream in 11 of the 17 cases in which the blood was cultured and in the fluid of the joint in the 2 cases in which the fluid was cultured.

Agglutinins were present in the blood of the infected persons but absent in the controls.

Cutaneous reactions to killed suspensions were present in 83 per cent of the patients tested late in convalescence, although absent in the controls.

Although crippling may be marked for a time, recovery tends to occur in from one to two months, with a small number of patients having persistent joint symptoms. No fatalities occurred.

EFFECT OF THEOBROMINE ON PERIPHERAL VASCULAR DISEASE

CLINICAL OBSERVATIONS

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For a number of years the purine-base group of drugs has been widely used in the treatment of angina pectoris. The fact that the symptoms of this disorder can often be relieved for long periods of time by these drugs [1] seemed to indicate that they might be useful in the treatment of peripheral arterial disease.

Dock had noted that the pain of intermittent claudication was sometimes relieved by theobromine,[2] and in the reports of the Council on Pharmacy and Chemistry of the American Medical Association it is stated that claims for the occasional relief of pain by theobromine and theophylline preparations in angina and similar lancinating pains may be permitted.[3]

Diseases of the peripheral arteries also offered the opportunity of estimating to some extent, at least, objective evidence of improvement as well as subjective relief of symptoms. The objective criteria employed were the simple ones usually used in diagnosis and consisted of the appearance of the affected part, such as the change in color at a constant level and with a change in position, the presence or absence of swelling and the degree of integrity of the tissues. Other objective measures used were demonstration of the condition of the superficial vessels by palpation and inspection and the measurement of the surface temperature.

The last-mentioned criterion was thought to be fairly reliable and to furnish the opportunity for some quantitative estimation. For this purpose determinations of cutaneous temperature were made in all of the cases by means of a thermo-electric couple of the common type. The results of observation were then compared and correlated with the subjective symptoms.

From the Medical Services of St. Luke's and Cook County Hospitals.

1. Gilbert, N. C., and Kerr, J. A.: Clinical Results in the Treatment of Angina Pectoris with the Purine-Base Diuretics, J. A. M. A. **92:** 201 (Jan. 19) 1929.

2. Dock, W.: The Use of Theobromine for Pain of Arteriosclerotic Origin, California & West. Med. **25:**636 (Nov.) 1926.

3. Reports of the Council on Pharmacy and Chemistry: Therapeutic Claims for Theobromine and Theophylline Preparations, J. A. M. A. **94:**1306 (April 26) 1930.

Observations were made under uniform conditions of room temperature, posture of the patient and rest. The locations at which temperature readings were taken were always the same. Readings were recorded only after the patient had been in the room for a minimum period of one-half hour or until the temperature readings became constant. Air currents were eliminated as much as possible. No attempt was made to control humidity, but it is believed that the conditions for dispersion of heat were uniform.

The apparatus was demonstrated to be accurate to 0.1 degree C. (0.18 degree F.), but no attention was paid to changes of less than 0.5 degree C. (0.9 degree F.), which allows a reasonable limit for error.

Early in the course of this work several drugs were used, including caffeine, theophylline [4] and others, but the best results seemed to be obtained with theobromine or theobromine sodium-acetate, and it is only the results with these preparations which are being reported.

The clinical material studied could be divided into three groups. These were arteriosclerosis with symptoms of intermittent claudication or with impairment of integrity of the tissues; thrombo-angiitis obliterans, presenting the same symptomatology, and functional disturbances, including Raynaud's disease and acrocyanosis.

In the functional group, no improvement could be shown. In one typical case of Raynaud's disease, study of the effect of theobromine therapy over a period of six weeks showed no subjective improvement, and temperature readings showed a wide fluctuation from day to day. The only conclusion to be drawn was that theobromine was without effect in improving this condition.

Several cases of acrocyanosis were similarly studied. No improvement could be demonstrated. The red-blue color and puffy swelling of the hands in all of the cases were unchanged, and no rise in cutaneous temperature could be demonstrated even after prolonged administration of the drug.

In the groups of arterial diseases, the results were much more promising, but not all were successful. The following cases have been selected as typical.

REPORT OF CASES

CASE 1.—A white woman, aged 74, entered St. Luke's Hospital complaining of constant severe pain in the right foot and toe, which was worse when the foot was dependent. It was impossible for her to walk. About a week before she had noticed swelling of the toe and a dusky red color; she thought that there was an infection about the nail. The pain continued and was often worse at night, sometimes assuming a cramplike character in the foot. During the previous winter she had noted difficulty in keeping her feet warm; the right one seemed

4. Also theophylline made soluble by ethylenediamine.

particularly affected. She had previously been under observation in the cardiac clinic for arteriosclerotic disease of the heart and auricular fibrillation.

Examination showed a well marked general arteriosclerosis of the senile type in a frail, small elderly woman. The heart showed moderate enlargement and auricular fibrillation which was well compensated without digitalis at the time. The right foot was cold and bluish red, and the great toe dusky. There was a marked biphasic color reaction, with pallor on elevation. The toe showed puffy swelling; the nail was loose, and there was a seropurulent discharge from the nail bed. There were no arterial pulsations palpable in either foot. The right foot was much colder than the left. Roentgen examination of the feet showed marked calcification of the vessels of both feet.

This patient was under close observation in the hospital from May 21 to September 4, and then in the outpatient department until April 16, of the following year. Frequent cutaneous temperatures were recorded.

TABLE 1.—*Arteriosclerosis with Ulceration of the First Toe of the Right Foot and Loss of the Nail (Case 1)**

Period of Observation	Average Temperature, C.		Appearance and Symptoms
	Right	Left	
Control, 17 days.....	28.9	31.4	Color change marked; toe painful
Theobromine sodium-acetate, 3 Gm. daily for 21 days	31.29	33.19	Pain less severe; color better; toe improving
Theobromine sodium-acetate discontinued for 15 days	30.2	32.6	Much better; toe nearly healed
Sodium acetate, 3 Gm. daily for 12 days	30.2	32.4	No pain; able to walk
Theobromine (alkaloid), 1 Gm. daily for 19 days	31.1	32.9	
Theobromine discontinued for 10 days	30.1	32.4	Foot in good condition; new nail grown; patient discharged taking 2 Gm. of theobromine sodium-acetate daily

* There was no medication other than that recorded except codeine for the relief of pain. The patient was confined to bed. Temperatures were taken on the dorsal surface of the feet proximal to the first toe.

During a control period of seventeen days the average temperature taken on the dorsal surface of the feet proximal to the first toe was 28.9 C. (84 F.) for the right and 31.4 C. (88.5 F.) for the left. During this time the foot looked bad, and the patient required sedatives for the relief of pain. Theobromine sodium-acetate was begun on June 7 (3 Gm. daily divided into three doses and continued for twenty-one days). During this period the average temperature rose to: right, 31.29 C. (88.32 F.); left, 33.4 (92.1 F.). There was still pain, but it was less severe, and by June 23 the patient was comfortable; no sedative was necessary, and the color of the foot looked better. No other treatment was used except to protect the foot from pressure. During this period the maximum temperature was 32 C. (89.6 F.) for the affected foot, a rise of 3.1 degree C. (5.6 degrees F.) above the control average.

Theobromine was discontinued from July 2 to July 17, with the average temperature falling to: right, 30.2 C. (86.3 F.) and, left, 32.6 C. (90.6 F.). Although the foot continued to improve, medication was continued again with a slight uniform rise and fall following its cessation. By July 29, the patient was able to walk and had no pain at night. The foot still showed change in color and loss of heat. By September 4, a new nail had grown; there was no discomfort, and

the patient was discharged taking theobromine sodium-acetate, 2 Gm. daily, at intervals, with no gastric distress. In February of the next year, pain returned, and observations were renewed for three months. I was again able to show a corresponding rise in temperature and clinical improvement during theobromine therapy for a time, but later complete relief was impossible. Coronary thrombosis finally caused death.

As shown in table 1, sodium acetate was substituted for the theobromine salt for twelve days following a period of no medication. There was no rise in temperature during this period.

CASE 2.—A white man, aged 59, said that he was able to walk slowly only about one-half block. He had a severe cramplike pain in the calf of the left leg, which forced him to stop for a few minutes, after which he was able to walk again. He had coronary thrombosis two years before and angina pectoris until recently, when precordial pain disappeared and auricular fibrillation set in. He had been having intermittent claudication for about three months. There was no impairment of integrity of the tissues; the dorsalis pedis and posterior tibial arteries

TABLE 2.—*Arteriosclerosis with Intermittent Claudication (Case 2)* *

Period of Observation	Average Temperature, C.		Appearance and Symptoms
	Right	Left	
Control, 5 days.....	30.1	27.9	Able to walk less than 1 block without pain
Theobromine sodium-acetate, 3 Gm. daily for 7 days	31.8	30.9	No claudication walking about ward; discharged to outpatient department
Theobromine sodium-acetate, 2 Gm. daily for 14 days	31.6	30.9	Walked 4 blocks without pain; feet hot and burning at night
Theobromine sodium-acetate, 2 Gm. daily for 30 days	....	....	Some aching in the left leg; able to walk 3 blocks; feet warm at night

* Temperatures were taken on the plantar surface of the first toes. The room temperature was 25.5 C.

could not be palpated on the left foot but were good on the right. There was definite loss of heat. During a three day control period, cutaneous temperature taken over the base of the first toe showed an average for the left foot of 27.9 C. (82.2 F.) and for the right, 30.1 C. (86.1 F.). Theobromine sodium-acetate was given, 3 Gm. daily, with a rise to a maximum temperature of 31.6 C. (88.8 F.) on the affected foot, which nearly equaled that on the other. The average for a period of eight days on this dose was 30.9 C. (87.6 F.), an average rise of nearly 3 degrees C. (5.4 degrees F.). At this time the patient was discharged from the hospital. He had no pain when walking about the ward and was able to walk four blocks without stopping. Because of gastric distress the dose was reduced to 2 Gm. daily, and observations were continued at weekly intervals. The increase in temperature was fairly well maintained until the patient discharged himself. He was able to walk five or six blocks as rapidly as cardiac dyspnea allowed him to. During the period of maximum dosage he complained of a burning sensation at night in the feet and legs which has been a common complaint when rather large doses are being taken.

CASE 3.—A woman, aged 60, with senile arteriosclerosis and varicose veins complained of pains in the legs, swelling, discoloration and tenderness. She could not sleep well at night because of the pain in the legs, which was more or less constant, and she was unable to walk because of weakness. She had been under

observation in the cardiac clinic for arteriosclerotic disease of the heart and auricular fibrillation. The feet showed marked varicosities, a dusky reddish-blue color, puffy swelling and pigmentation. Arterial pulsations could not be palpated. There was no impairment of the integrity of the tissues. Roentgenograms showed marked calcification of the vessels in the legs. There was definite loss of heat, about the same in both feet. During a twenty-five day period of observation no subjective improvement could be shown, nor was there an increase in surface temperature, which fluctuated within a moderate range.

CASE 4.—F. H., aged 42, a white man with thrombo-angiitis obliterans, complained of intermittent claudication. Left lumbar sympathectomy had been performed one year previously by Dr. Loyal Davis. Following lumbar sympathectomy the symptoms of Buerger's disease in the left leg and foot had disappeared, and he had been able to work without discomfort until about two months before, when he began to have pain in the calf of the right leg when walking. The foot became cold and showed a dusky discoloration. The patient was unable to walk more than two blocks without being required to rest, after which the cramplike

TABLE 3.—*Arteriosclerosis and Varicose Veins (Case 3)**

Period of Observation	Average Temperature, C.		Appearance and Symptoms
	Right	Left	
Control, 7 days.....	26.7	27.0	Feet cold and mottled; veins dilated; weakness and aching in legs during walking
Theobromine sodium-acetate, 3 to 3.7 Gm. daily for 11 days	24.6	24.5	No change in appearance or symptoms
Theobromine sodium-acetate stopped for 5 days	25.0	25.1	No change
Theobromine sodium-acetate, 2.7 Gm. daily for 7 days	24.8	24.8	No subjective or objective change

* The patient was hospitalized and kept in bed for the first period of therapy. Temperatures taken on the plantar surface of the first toe remained constantly below the average room temperature of 25.5 C.

pain would stop and he could continue. Roentgenograms showed little evidence of arteriosclerosis. The left leg and foot were warm and dry, and perspiration was not evident. The color was pink and the circulation appeared to be good. The dorsalis pedis artery was faintly palpable. The right foot was cold and moist and showed a dusky, bluish-red color of the toe when the foot was dependent, which changed to white when the foot was elevated. There were no arterial pulsations to be detected below the popliteal artery. The patient was under observation for four months. During the initial control period the surface temperature averaged 28.6 C. (83.4 F.) on the dorsal surface proximal to first toe; that of the toe itself averaged 27 C. (80.6 F.). The readings over various areas on the left foot were about equal, with an average of 31.8 C. (89.2 F.) on the toe. The surface temperature on the dorsum of the foot after ten days during which theobromine sodium-acetate was administered reached 32.6 C. (90.68 F.), the maximum for the period of administration, which covered thirty-three days, with the dosage varying from 2 to 3 Gm. daily. During the first ten days the patient was able to walk five blocks; pain at night had nearly disappeared. The maximum distance he was able to walk was six blocks. With the higher dosage he also complained of a severe burning sensation in the affected leg at night. The sympathectomized leg showed a smaller increase in temperature, reaching a maximum of 1.7 degrees C. (3 degrees F.) above the average, and with an average increase for

the period of 0.6 degree C. (1 degree F.). With cessation of therapy there was a fall in temperature on the affected foot to approximately the control levels, but symptoms of intermittent claudication did not return to the previous degree of severity for thirty-four days, although the distance the patient could walk was gradually diminished.

With restoration of therapy there was again a rise in temperature, and the patient was able to walk five blocks.

CASE 5.—Charles T., a white man, aged 53, with thrombo-angiitis obliterans, entered St. Luke's Hospital on March 1, 1930, with a history of pain in the right foot and toe for six months. Pain was constant day and night, requiring sedatives daily and morphine at night. The foot was badly discolored and cold, with beginning gangrene involving the first toe. The arteries of the feet were not

TABLE 4.—*Thrombo-Angiitis Obliterans with Previous Left Lumbar Sympathectomy (Case 4)**

Period of Observation	Average Temperature, C.		Appearance and Symptoms
	Right	Left	
Control period, 2 days.....	27.0	32.0	Intermittent claudication; unable to walk 2 blocks at slow gait without pain; typical color reactions; arterial pulsations absent
Theobromine sodium-acetate, 3 Gm. daily for 36 days	29.6	32.8	Able to walk from 5 to 6 blocks slowly without pain even in cold wet weather; complained of burning sensations in the legs, worse in the right
Theobromine sodium-acetate discontinued for 34 days because of burning sensation at night	27.4	31.6	Comfortable for a time but at end of period pain returned; 2 blocks the limit when walking without stopping for rest
Theobromine sodium-acetate, 3 Gm. daily for 22 days	30.0	31.2	Improved as before; color better; able to walk about 5 blocks, but not entirely without pain

* Temperatures were taken on the dorsal surface of the feet proximal to the first toe. The room temperature was 25.5 C. This patient complained of severe burning sensations in both legs during the periods of administration of theobromine. The individual temperature readings showed more fluctuation than was usual, possibly because the patient was under observation during cold winter weather.

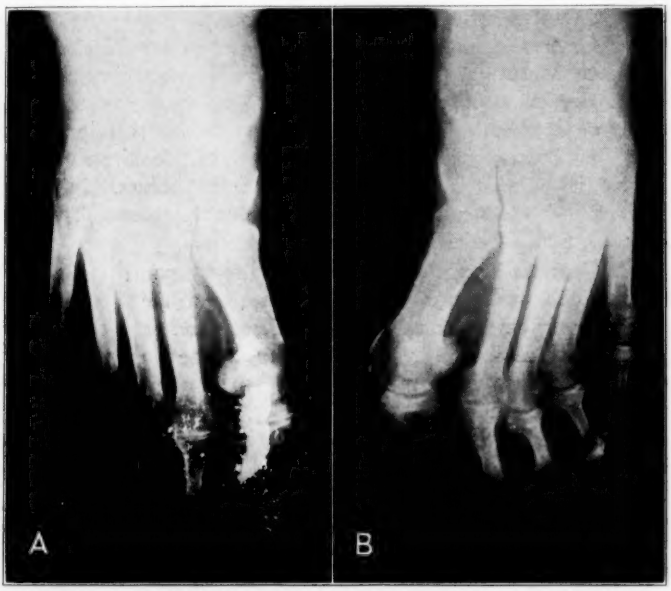
palpable, and roentgenograms showed no evidence of calcification. The patient could not walk. Theobromine sodium-acetate was without favorable response. There was no subjective relief or a rise in surface temperature. It was impossible to demonstrate vasodilatation by foreign protein fever or by spinal anesthesia. It was therefore assumed that there was complete occlusion of the peripheral arteries of the foot.

CASE 6.—H. A., aged 33, a white man with thrombo-angiitis obliterans, entered Cook County Hospital complaining of pain in the right foot and great toe. There were swelling and discoloration of the toes of the right foot, with beginning gangrene in the first toe. The illness was of thirteen days' duration, following an alcoholic debauch. Roentgenograms showed no calcification of the vessels. The changes in color were well marked. The arteries were not papable. It was impossible to maintain uniform room temperatures in this case, and the cutaneous temperatures showed less uniformity of response. There was an initial rise from the control level of 29.8 C. (85.6 F.) on the affected side and 32.3 C. (90.1 F.) on the left to a maximum of 31.6 C. and 33.2 C. (91.7 F.) respectively. The average for the period of observation was 31.5 C. (88.7 F.) for the affected foot

and 32.8 C. (91 F.) for the other. There was no appreciable fall after theobromine was discontinued, during the period of observation. Relief from pain was prompt and complete. The foot improved remarkably in appearance, and a line of demarcation formed which was distal to what was expected before this therapy was instituted. The toes were amputated later, and the wounds healed, although slowly. The patient left the hospital without complaint of pain.

COMMENT

The material presented has been selected from a group of cases observed during the past five years. For many of these cases complete



Roentgen appearance of the vessels in case 1. In the left or unaffected foot (*A*) the artery appears to be completely calcified, while in the right foot (*B*) there is only patchy calcification. This foot was colder than the left and painful, and showed loss of integrity of tissue in the first toe.

data have not been recorded, but the clinical results of the use of theobromine and its salts in conjunction with other forms of treatment in arteriosclerotic disease of the peripheral arteries and thrombo-angiitis obliterans have been sufficiently favorable to demonstrate its value.

It is particularly applicable to cases of arterial disease in the aged, in whom more energetic methods are impossible. As in case 1, a variable amount of relief may be provided over rather long periods of time, if there is sufficient capacity for dilatation of the affected vessels.

It is interesting to note that in this case roentgenograms demonstrated only a patchy calcification in the vessels of the affected foot, while in the other foot, which was symptom-free, the calcification was more nearly continuous (figure). It might be supposed that under such conditions a vessel the wall of which was completely calcified would be incapable of constriction or dilatation, while, on the other hand, a patchy sclerosis might result in increased irritability and consequently angiospasm with ischemia to the tissue supplied. It is in cases of this type that benefit from theobromine may be expected.

In thrombo-angiitis obliterans a similar set of conditions may exist. In early cases in which occlusion is not too extensive and in which there is a large element of angiospasm, theobromine given in sufficient amounts has been successful in bringing symptomatic relief as well as restoration of minor degrees of impairment of tissues. This has been observed particularly in instances of involvement of the upper extremities. Case 5 clearly shows that when organic impairment of the larger vessels is extensive, no objective or subjective improvement occurs. In this case it was impossible to demonstrate an element of angiospasm. The failure in case 3 can be explained on a like basis.

It is, however, unfortunately true that many patients in whom a good response was expected failed to improve. This has been particularly true in cases of diabetic arteriosclerosis and gangrene. In none of these cases has it been evident that theobromine was responsible for improvement that could not be accounted for by proper control of the diabetes and the complicating infection.

The mode and location of the action of these drugs can only be surmised. The fact that they are without effect in purely functional disorders, as in Raynaud's disease, and are most active in disease of the medium-sized and smaller arteries, where an element of angiospasm may exist, suggests that the effect may be a local one, possibly that of reducing the increased irritability of the musculature of the diseased vessel wall. This interpretation is in agreement with the views of Sollmann and Pilcher, who stated that caffeine is a vasodilator acting by peripheral inhibition of vasoconstriction.[5] It is likewise interesting to note that the coronary arteries are of the same class of vessels.

CONCLUSIONS

1. Theobromine and its salts, particularly theobromine sodium-acetate, act as peripheral vasodilators.

2. They are useful in the treatment of peripheral arteriosclerosis and early cases of thrombo-angiitis obliterans in which there is a large ele-

5. Sollmann, T., and Pilcher, J. D.: The Actions of Caffein on the Mammalian Circulation, J. Pharmacol. & Exper. Therap. **3**:19, 1911.

ment of angiospasm. Their use results in subjective improvement in intermittent claudication, and repair of loss of tissue integrity has been noted.

3. They are not effective in all cases, but the results obtained with them are sufficiently good to make them a valuable adjunct in the treatment of peripheral vascular diseases.

CHANGES IN THE CENTRAL NERVOUS SYSTEM RESULTING FROM CONVULSIONS DUE TO HYPERINSULINISM

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A series of experiments with animals was designed to investigate whether or not repeated convulsions for varying periods of time, induced artificially by the production of hyperinsulinism, would lead to organic lesions in the brain. Convulsions are generally classified as organic when there are associated with them demonstrable intracranial pathologic conditions, and as functional when no such evidence exists.

It is still a question whether convulsions per se lead to definite organic cerebral lesions. Spielmeyer [1] showed that focal anatomic lesions of the necrobiotic type in the brain can result from a purely functional circulatory disturbance. Furthermore, he noted similar lesions associated with convulsions in a variety of intoxications, such as eclampsia of pregnancy, pseudo-uremia, carbon monoxide intoxication, chloroform narcosis and infections, in none of which there was any organic occlusion of a cerebral vessel. All of these he regarded as due to vasomotor disturbances.

Foster Kennedy, in a discussion of Spielmeyer's paper,[1b] described the occurrence of marked cerebral ischemia during an epileptic fit. This clinical observation was made by him on a patient whose brain was exposed during an operation under local anesthesia.

The importance of this subject, and the meagerness of knowledge concerning the effects on the central nervous system of convulsions of varying intensity, duration and frequency, led us to undertake this study.

PROCEDURE

Fourteen rabbits, weighing from 2 to 5 pounds (0.9 to 2.3 Kg.), were employed in this study and were fed the regular stock diet. Convulsions were produced by the intravenous injection of insulin into animals which had previously fasted for eighteen hours. The dosage of insulin required to produce an attack varied considerably (from 2 to 12 units) for different rabbits. In certain instances it was found necessary during the course of the experiment to increase the dose to bring about an attack. The convulsions, as a rule, set in from two to six hours after injection of the insulin. The animals were allowed to remain in convulsions for

From the Department of Pathology, Yale University School of Medicine.

1. Spielmeyer, W.: (*a*) J. Ment. Sc. **76**:641, 1930; (*b*) J. Nerv. & Ment. Dis. **71**:293, 1930; (*c*) Anatomic Substratum of Convulsive State, Arch. Neurol. & Psychiat. **23**:869 (May) 1930.

periods ranging from three minutes to several hours. The convulsions were then terminated by the intravenous administration of 10 cc. of 50 per cent dextrose. Two or three days were allowed to elapse between successive injections of insulin into the same animal. Whenever possible the experiment was conducted over a period of about three months. At the close of the experiments the animals were killed with ether, and autopsy was performed within two hours. Blocks of tissue for histologic examination were taken from the medulla, pons, cerebellum, basal ganglions and frontal, parietal and temporal lobes. They were fixed in 95 per cent alcohol and stained with toluidine blue by Nissl's method. The lesions were arbitrarily graded from — to + + + +, depending on the severity of the changes noted in the preparations.

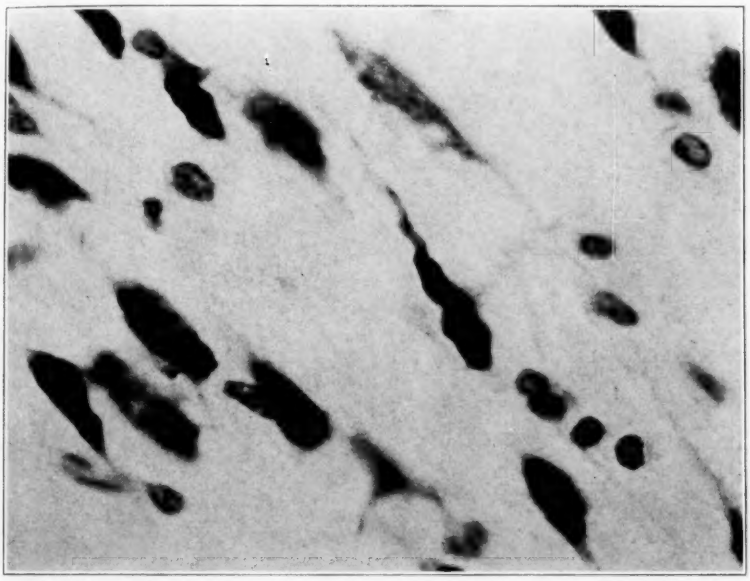

Fig. 1.—A shrunken hyperchromatic ganglion cell showing a corkscrew process staining for a long distance. Nissl stain; × 750.

RESULTS

Gross inspection of the brains at necropsy revealed no changes. Both hemispheres were symmetrical; the convolutions and sulci, respectively, were of the usual width and depth. There were no large or petechial hemorrhages and no edema. The microscopic pictures, which varied with the severity of the lesions, were graded from — to ++++ by the following criteria:

— No abnormal cells were found in any of the preparations.

—+ Only occasional shrunken, hyperchromatic cells with corkscrew processes that stained for long distances were seen scattered among the nerve cells of the third and fifth cortical layers. Figure 1 is a photomicrograph of such a cell.

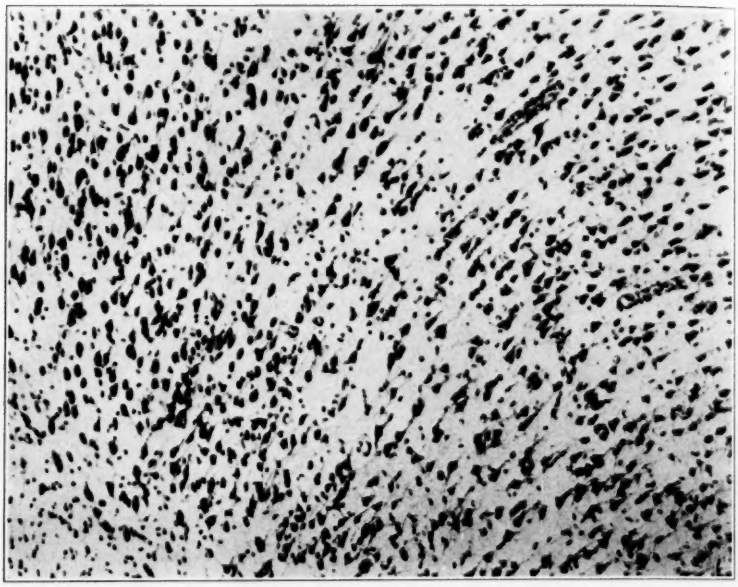

Fig. 2.—A zone showing many shrunken cells with corkscrew processes. Nissl stain; × 125.

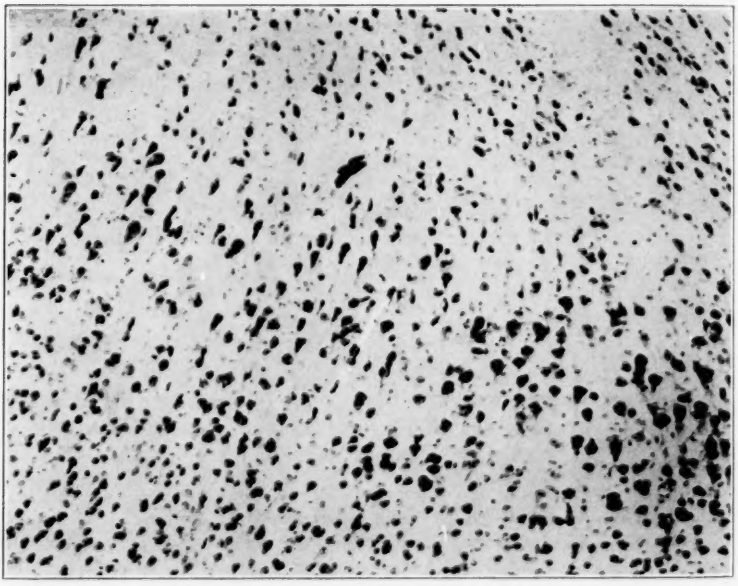

Fig. 3.—A zone of necrobiosis in which the cells stain poorly and faintly or are completely gone. Nissl stain; × 125.

\+ Definite but disseminated small zones showing these cells were found in the cerebral cortex.

++ Many more such zones were present, and each contained many more shrunken, hyperchromatic cells. Figure 2 is a photomicrograph of such a zone.

+++ In addition to these zones, areas of necrobiosis were present in which many cells were completely absent or poorly stained. Figure 3 is a photomicrograph of such a zone.

++++ The zones of necrobiosis were more extensive, and there was in addition a marked glial proliferation in these regions. Figure 4 is a photomicrograph of such a zone. Few nerve cells and many glia cells are seen in this figure, as contrasted with figure 5, which is from a comparable

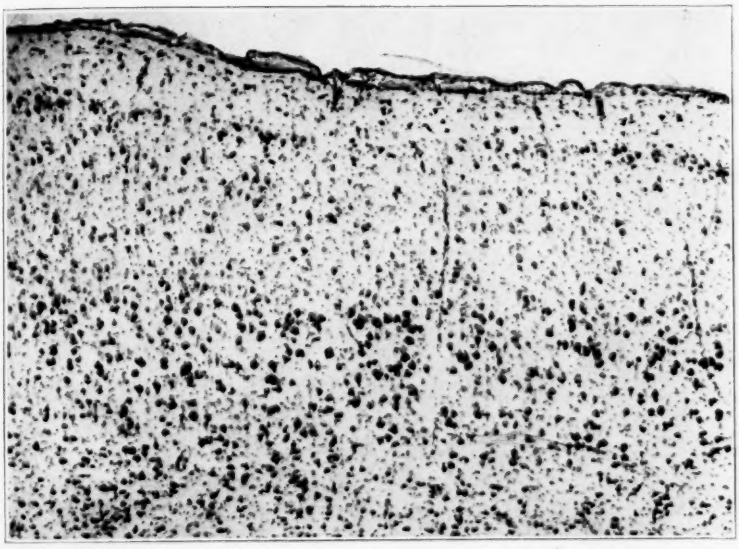

Fig. 4.—A zone of necrobiosis, showing more extensive lesion with marked glial proliferation. Nissl stain; × 75.

normal region under the same magnification from the opposite side of the same animal. Figure 6 shows more clearly under higher magnification the type of cells present in the necrobiotic area shown in figure 4.

The results of these experiments are shown in the table. Rabbit 4 received thirteen injections of 1½ units of insulin, and at no time manifested either convulsions or tremors. On the twenty-second day encephalitis set in. The animal was killed and autopsy was performed the same evening. Examination of the brain showed only the lesions of encephalitis. Rabbit 3 received thirty-four injections of 2½ units of insulin over a period of one hundred and four days and only once mani-

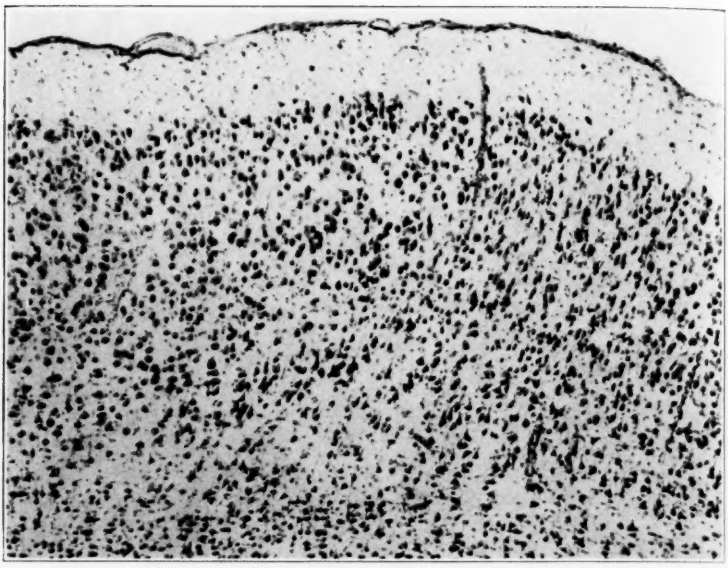

Fig. 5.—A normal area comparable to that in figure 4 from the opposite side of the same animal. Nissl stain; × 75.

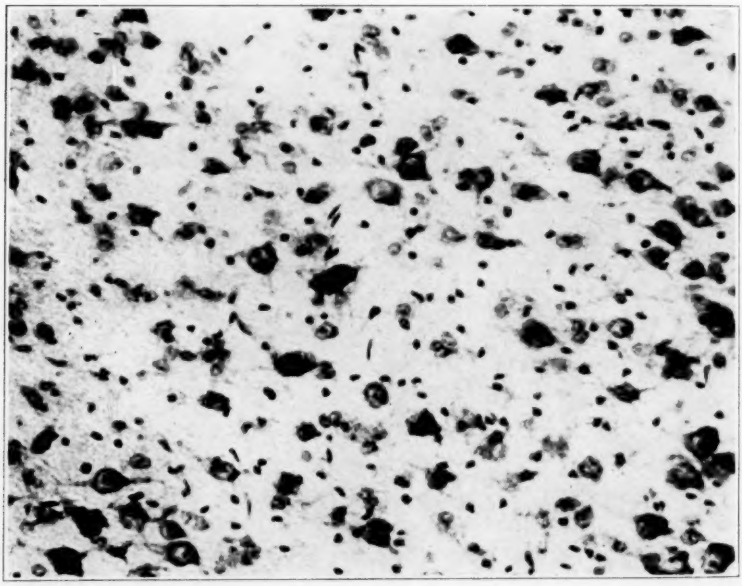

Fig. 6.—A portion of the zone in figure 4, showing the type of cells. Many glia cells and a few nerve cells are seen. Nissl stain; × 500.

fested tremors for thirty minutes. There were no pathologic changes in the brain. Rabbits 5, 1 and 9 showed only minimal changes in the brain. Of these, rabbit 5, inoculated thirty-one times during the course of one hundred and five days, displayed two slight convulsions lasting three and thirty minutes, respectively. Rabbit 1 received four injections over a period of ten days and had two severe convulsions, during the last of which it suffered a fracture of the spine, with resulting paralysis of the hindlegs. Rabbit 9 was given twenty-one injections, and with the dosage employed (from 4 to 6 units) it was possible to

Insulin Dosage, Duration and Severity of Convulsion and Extent of Lesion

Rabbit	Weight, Lbs.	Insulin, Units	No. of Injections	Convulsions			Comment	Duration of Experiment, Days	Microscopic Grading
				Slight	Severe	Duration			
4	2⅜	1.5	13	..	..	.....	Encephalitis set in	22	—
3	5	2.5	34	1	..	30 min.	.....	104	—
5	2	2	31	2	..	3 and 30 min.	.....	104	— +
1	2¼	2	4	..	2	4 hrs.	Broke spine; paralysis of hindlegs	10	— +
9	4¾	4-6	21	11	1	15 min.	Difficult to produce convulsion	77	— +
6	2⅞	2	2	..	1	5 hrs.	Broke spine; paralysis of hindlegs	6	+
8	4¼	3	1	..	1	4 hrs.	Very severe convulsion; died next day	1	++
2	3⅜	2	8	..	7	6-3 min. 1-4 hrs.	.....	20	++
7	3⅜	3-5	25	8	12	9-3 min. 3-20 min.	.....	105	+++
10	4	4-6	17	1	12	8-4 min. 4-20 min.	.....	77	+++
11	3¾	4-6	19	3	9	5-4 min. 4-20 min.	.....	76	+++
14	4½	8	15	..	15	8-2 hrs. 7-1½ hrs.	.....	84	+++
13	4½	8-10	18	..	14	14-2 hrs.	Showed encephalitis histologically	85	++++
12	4½	8-12	22	4	12	8-2 hrs. 4-1½ hrs.	.....	86	++++

produce only one severe convulsion lasting fifteen minutes. However, the animal did display tremors on eleven other occasions. The other rabbits (6, 8, 2, 7, 10, 11, 14, 13 and 12), all of whom had severe convulsions one or more times for variable periods of time, showed definite anatomic lesions, as seen in the table. Rabbit 14 showed many small foci of necrobiosis of the cortical ganglion cells and many more shrunken cells than did the preceding animals. It did not, however, show the extensive zones of necrobiosis and the glial proliferation in these regions seen in rabbits 13 and 12. Rabbit 12 showed marked glial proliferation in the extensive necrobiotic areas. Many shrunken cells were also found scattered throughout all the preparations of this animal. Rabbit 13 showed lesions similar to those formed in the preceding animal. In addi-

tion, however, it showed the exudative lesions of spontaneous encephalitis, although clinically it manifested no symptoms of this disease.

The lesions of spontaneous encephalitis in rabbits cannot be confused with those found in these experiments. The former are of an exudative character, and consist of focal accumulations of small round cells, with some edema and necrosis. They do not cause the changes in the pyramidal cells noted here, nor are they characterized by necrobiotic changes.

COMMENT

It is quite apparent from these results that animals which have not had convulsions, or only slight ones on a few occasions, show either minimal or no cerebral changes, regardless of the number of injections of insulin they have received. However, even one convulsion, if prolonged and severe enough, may produce definite lesions in the central nervous system. Furthermore, the more prolonged or the more severe the convulsions, the more extensive are the lesions found, provided the animals are permitted to live long enough for the changes to develop. Popper and Jahoda [2] expressed the belief that insulin contains a component which directly affects the central nervous system so as to produce convulsions. They base this opinion on their own work and the work of others in which the convulsive effects of insulin were neutralized by the administration of caffeine, liquid petrolatum and other substances without in any way affecting the blood sugar. Others feel that the convulsions may be due to cerebral anemia. Several workers noted the fact that anoxemia of the brain, if prolonged and severe, is regularly followed by convulsions. Gildea and Cobb [3] described lesions very similar to those seen in our experiments which they produced by ligating the carotid arteries of cats. What the exact mechanism is which produces the cerebral lesions in the rabbits cannot be stated definitely, but it seems to me that during the convulsions some circulatory disturbance takes place, with consequent anoxemia of the brain. During the more severe convulsions, these periods of anoxemia are sufficiently prolonged to cause the cerebral changes.

SUMMARY

1. Fourteen rabbits were employed in these experiments.

2. Rabbits which did not have convulsions showed no lesions in the brain.

3. Rabbits which had slight convulsions showed minimal or no changes in the brain.

2. Popper, L., and Jahoda, S.: Klin. Wchnschr. **9**:1585, 1930.

3. Gildea, E. F., and Cobb, S.: Effects of Anemia on Central Cortex of Cat, Arch. Neurol. & Psychiat. **23**:876 (May) 1930.

4. Rabbits which had severe convulsions one or more times showed definite anatomic lesions in the central nervous system.

5. The greater the number and the more prolonged the convulsions, the more severe were the lesions.

6. The milder lesions consisted of zones containing shrunken hyperchromatic cells with corkscrew processes. The more severe lesions showed, in addition, zones of necrobiosis. The cells particularly involved were the pyramidal cells of the third and fifth cortical layers.

HEPATITIS AND CHOLECYSTITIS IN THE COURSE OF BRUCELLA INFECTION

REPORT OF A CASE

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AND
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Brucella infection in man is a relatively common disease in the United States, as shown by the increasing number of reports from all parts of the country. In the numerous clinical reports on the subject there is, however, relatively little to be found concerning the visceral manifestations of the disease in man. Clinically, in a few reported cases abdominal pain has been the predominant symptom and has led to the consideration of cholecystitis or appendicitis as the possible diagnosis. Simpson [1] has recorded twelve appendectomies and two cholecystectomies performed on patients with undulant fever. In two of the cases studied by Hardy and his associates [2] a diagnosis of cholecystitis was seriously considered. Leavell and Amoss [3] reported a case of brucelliasis in a patient whose illness was complicated by cholecystitis, and from whose bile they were able to isolate Brucella organisms. In their article they include the case reported by Bull and Gram in 1911. In addition, Amoss [4] studied two other patients with brucelliasis on whom cholecystectomies were performed.

The following case is recorded on account of its rarity, certain unusual features in the clinical picture and the relief afforded the patient by operation. The patient had an illness of several months' duration characterized by leukopenia and an undulating fever.

REPORT OF A CASE

History.—W. O., an American, aged 57, married, an animal caretaker, was admitted to the University of California Hospital on Feb. 12, 1931, complaining of weakness, cough and fever. He had been well until three weeks before

From the Division of Medicine, Medical School, and Hooper Foundation, University of California.

1. Simpson, W. M., and Bowers, L. G.: Surgical Aspects of Undulant Fever, Am. J. Surg. **7**:597, 1929.

2. Hardy, A. V.; Gordon, C. F.; Borts, F. H., and Hardy, G. C.: Undulant Fever with Special Reference to Study of Brucella Infection in Iowa, Bull. Nat. Inst. Health no. 158, 1930.

3. Leavell, H. R., and Amoss, H. L.: Brucella Infection: Case Report; Cultivation of Brucella from Bile, Am. J. M. Sc. **181**:96, 1931.

4. Amoss, H. L.: Localization of Brucella, Internat. Clin. **4**:93, 1931.

admission, when he was seized with a severe, sharp pain in the right side of the chest, accompanied by a "shaking" chill, headache, cough and a temperature of 104 F. His family physician the following day made a tentative diagnosis of bronchopneumonia. Five days after the onset of his illness the patient became irrational, restless, talkative and delirious. For six days he had several severe chills daily and a temperature in the afternoons of 40 C. (104 F.). There was no appreciable change in his condition after he had been in bed three weeks. He remained semicomatose and became incontinent of urine and feces. When the patient failed to improve, he was referred to the hospital.

The past history was unimportant except that he had had typhoid fever when 20 years of age. He had lived in California for the past thirty-seven years as an animal caretaker in an experimental laboratory where he came indirectly in contact with animals that had been experimentally infected with Brucella abortus and Brucella melitensis. It is known that he had assisted his co-workers in the animal house during the butchering of a hog which had previously been inoculated with a virulent strain of Br. melitensis of caprine origin.

Physical Examination.—Examination showed a well developed and moderately well nourished man appearing extremely ill and in a semistuporous condition. The temperature was 39 C. (102.2 F.); the pulse rate, 92, and the respiratory rate, 22. The mucous membranes were slightly pale and the skin moist. Both eyes showed a marked arcus senilis. The ears, teeth, nose, throat and lymph nodes presented no remarkable abnormalities. There was a slight dulness, with some diminution of breath sounds and coarse, relatively dry râles at the base of the right lung posteriorly. There was no evidence of cardiac disease. There was a moderate generalized arteriosclerosis. The abdomen was somewhat distended but there was no tenderness, and no tumor masses could be felt. The external genitalia appeared normal; examination of the rectum showed a slightly enlarged prostate gland; the extremities were free from edema, and the tendon reflexes were normal.

Laboratory Procedures.—On admission the blood count showed: red blood cells, 4,250,000 per cubic millimeter; hemoglobin, 70 per cent (Sahli), and leukocytes, 4,120. The differential count showed: polymorphonuclear leukocytes, 81 per cent; lymphocytes, 15 per cent, and monocytes, 4 per cent. There appeared to be no other abnormality of the blood.

Roentgen examination of the chest failed to reveal any evidence of pulmonary disease.

The urine showed a faint trace of albumin. The reactions to the Wassermann and Kahn tests of the blood were normal.

On February 13, March 3, March 18 and April 3, blood serum failed to agglutinate the following organisms: Bacillus typhosus, Bacillus paratyphosus A, Bacillus paratyphosus B, Bacillus enteritidis and Bacillus coli. However, the agglutination tests with Br. abortus were as follows: On February 13, complete agglutination was obtained in a dilution of 1:320 and partial agglutination in a dilution of 1:640; similar results were obtained on March 4, 18 and 28. Intracutaneous injections of an extract from Brucella abortus were followed by hyperemia and accordingly were considered as giving positive reactions. Samples of venous blood obtained on numerous occasions during the height of the fever were reported as yielding no growth of organisms. A duodenal tube was passed, and when the olive was in place 15 cc. of clear bile was removed. Following this, 25 cc. of 50 per cent magnesium sulphate was injected and, after a free flow of bile was obtained, several specimens were procured by aseptic technic. These specimens failed to yield a growth of Brucella organisms following their application to suitable culture mediums. No unusual organisms were isolated

from repeatedly obtained specimens of urine or stools. A presumptive diagnosis of undulant fever was made, but the diagnosis could not be established with certainty.

Clinical Course and Treatment.—During the first month of the patient's stay in the hospital, there was some clinical improvement with a gain in strength. During this month, however, anemia developed in which the red cell count and hemoglobin reached a low level of 2,729,000 cells per cubic millimeter and 48 per cent (Sahli), respectively. On April 10 the patient was given an intravenous injection of 0.4 cc. of a lysed vaccine made from cultures of Br. melitensis and Br. abortus based on the method of Caronia.[5] On April 14, an injection of 0.1 cc was given. This treatment was discontinued because of the patient's anemia, and on April 20 he was given a transfusion of 265 cc. of whole blood. During the next few weeks there was marked clinical improvement in his condition. The anemia gradually decreased and the leukocytes returned to normal. Throughout his stay in the hospital, the patient's temperature was of the undulating type. Each undulation would slowly rise from about 37 C. (98.6 F.) to about 40 C. and then slowly decrease again to 37 C. Each cycle extended over a period of from eight to twelve days, during which time there was fever, alternating with periods of from two to four days of subnormal or normal temperatures. The undulations of fever gradually subsided, and after the temperature had remained normal for one month, the patient was discharged from the hospital on August 1, nearly six months after entry.

Second Admission.—The patient was readmitted on September 14.

History Preceding Second Admission.—Following his discharge from the hospital, the patient returned to his home for convalescence. Daily observations of his temperature showed it to be 37 C. or less, except for an occasional rise to 37.2 C. (98.9 F.). Four days before readmission to the hospital while recuperating in the mountains, he fell asleep in the sunshine and was exposed to the sun's rays for about one-half hour. The atmospheric temperature was about 104 F. When he awakened, he felt as though he were "burning up"; he had a severe headache and a temperature of 39 C. For the following three days he continued to feel ill, and his temperature remained elevated until he entered the hospital.

Physical Examination.—The patient did not appear extremely ill but was somewhat lethargic. The temperature was 39 C.; the pulse rate, 90, and the respiratory rate, 22. There was nothing new except a generalized maculopapular, erythematous rash. This rash, which we have observed in other patients with undulant fever, was neither irritative nor painful. The superficial lymph nodes were not enlarged. Neither the spleen nor the edge of the liver could be palpated.

Second Laboratory Examination.—Specimens of venous blood were removed on the day of entry and again on the following day. One of these was sent to the laboratory of the hospital and the other to Dr. Meyer's laboratory at the Hooper Foundation. Ten days later a report was received from each laboratory stating that Brucella organisms had been isolated from both specimens. This organism was later identified as Br. melitensis.[6] Other laboratory procedures

5. Caronia, J.: Nature and Uses of Lysed Typhoid Vaccine, Am. J. Dis. Child. **39**:1 (Jan.) 1930.

6. According to Dr. K. F. Meyer, Miss B. Eddie and Miss Veazie, the strain of Brucella isolated from W. O. gave the following reactions: positive stain with thionine (50,000), fuchsin and pyronine; reduction of nitrates; no formation of gas; no hydrogen sulphide; agglutinin absorption of Br. melitensis; low pathogenicity for monkeys and guinea-pigs; no febrile reaction in monkeys; infection in guinea-pigs with not less than 1,500 organisms.

revealed nothing of significance. A biopsy specimen from one of the lesions in the skin was taken. Cultures of this tissue failed to show any bacterial growth. Microscopic examination showed vascular hyperemia and a slight perivascular infiltration of lymphocytes consistent with a diagnosis of subacute inflammation of the skin.

Clinical Course and Treatment.—Twelve days after entrance into the hospital, the patient had a sudden attack of severe, constant pain in the right upper quadrant of the abdomen. There was marked hyperesthesia of the skin to the right of the midabdominal line and between the umbilicus and the right costal margin. There was spasm of the muscles in this region, and pain was elicited on deep palpation. A resistance resembling a cystic mass could be felt in the region of the gallbladder. A presumptive diagnosis of acute cholecystitis was made and the patient was afforded symptomatic relief. Morphine was administered and ice packs were applied locally. Specimens of bile were again removed from the duodenum, but these, as on previous occasions, failed to show bacterial growth on culture.

The patient was held under observation and given symptomatic relief until October 22, four weeks after the onset of the pain in the right upper quadrant. During this time the patient's symptoms referable to the region of the gallbladder had gradually subsided. One month after entry the rash disappeared. He was transferred to the surgical service on Oct. 22, 1931, about five weeks after entrance to the hospital.

On October 22, a cholecystectomy was performed by Dr. H. Glen Bell. The wall of the gallbladder was found to be thickened and there were adhesions binding it to the liver and adjacent duodenum. Several centimeters from the gallbladder in the left lobe of the liver, a small band of adhesions extended to the anterior parietal peritoneum. When this was cut through, a small round nodule about 1 cm. in diameter was seen in the parenchyma of the liver. This nodule and a surrounding margin of hepatic tissue were removed. The nodule was a rounded, white, fibrous structure with central softening and a granulomatous appearance. A small bit of semipurulent material was expressed and some of this was prepared for culture.

Microscopic Examination of Tissues (by Dr. A. H. Heald, Department of Pathology).—The sections of the gallbladder showed an eroded mucosa, largely replaced by vascular granulation tissue and diffusely infiltrated by lymphocytes and a few neutrophils and eosinophils. The wall was edematous, and inflammatory cells were infiltrated throughout the fibrous septums. Two small, early granulomatous patches were found, one having a small giant cell of the foreign body type at its center.

The sections from the liver showed a bizarre, destructive, inflammatory process in varying stages. The cellular reactions centered about a granulomatous process 15 mm. in diameter, the center of which consisted of puriform material, disintegrating polymorphonuclear leukocytes and cellular débris. This was surrounded by a vascular connective tissue wall, epithelioid cells and lymphocytes, among which might be seen an occasional small multinucleated giant cell. Beyond this central lesion there was an infiltration with lymphocytes and plasma cells about the portal channels with an inflammatory proliferation of the epithelium of the bile ducts. Some of the lesions were in an early stage and showed a few neutrophils and eosinophils in the triad spaces. In the intervening hepatic parenchyma, there were numerous small, discrete, tubercle-like foci which consisted of lymphocytes, epithelioid cells and an occasional small giant cell. All foci were surrounded by a proliferative reaction of fibrous tissue which tended to encapsulate them.

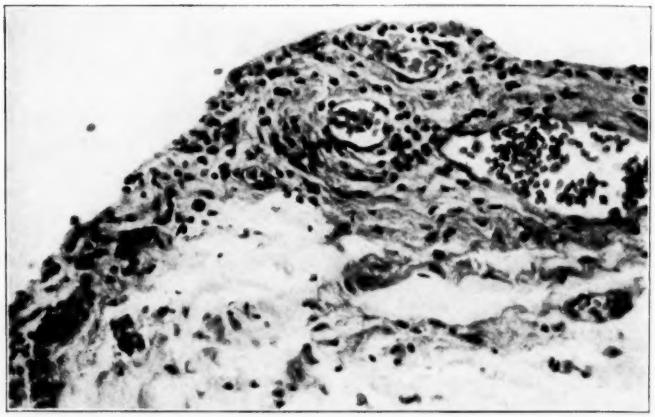

Fig. 1.—Section of the wall of the gallbladder showing increased vascularity and cellular infiltration.

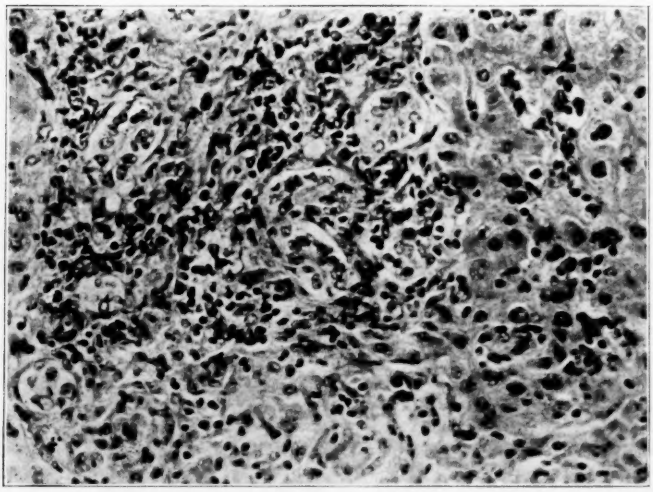

Fig. 2.—Cellular infiltration in the periportal connective tissue and swollen epithelial cells of the bile ducts.

Sections of the wall of the gallbladder and of the granulomatous tissue, fixed in Zenker's fluid, revealed numerous small, gram-negative rods occurring singly and in pairs. In the gallbladder myriads of such organisms were found in the mucosal granulation tissue and throughout the wall. They showed particularly well between the edematous smooth muscle bundles. In the liver the bacilli were found only in the smaller purulent inflammatory zones.

Cultures taken immediately after the removal of the tissues yielded a growth of Br. melitensis from both the submucosa of the gallbladder and the granulomatous process in the liver.

Following the operation, the patient's course was uneventful. His temperature returned to normal within a few days and remained so except for an occasional rise in the afternoon to 37.4 C. (99.3 F.). Cultures of blood, stool and a specimen

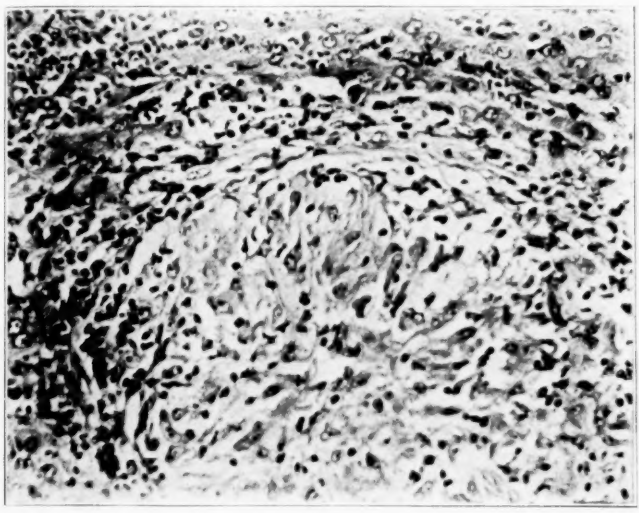

Fig. 3.—An early lesion in the hepatic parenchyma adjacent to the main granulomatous nodule. Note the tubercle-like arrangement of epithelioid cells, lymphocytes and fibroblastic tissue. High power.

of bile removed through a duodenal tube on November 23 failed to show a growth of Brucella organisms. The patient was discharged from the hospital on November 24. He has had no recurrence of symptoms of active brucelliasis up to the present time.

COMMENT

Clinical Features.—A diagnosis of this patient's illness was masked during the onset by signs and symptoms suggestive of pneumonia. These disappeared within three weeks' time, but the patient's condition continued to be grave and a high fever and leukopenia persisted. A diagnosis of typhoid and paratyphoid infection was practically excluded

by the laboratory observations. Bacterial endocarditis or other conditions frequently associated with bacteremia were considered unlikely in the absence of petechiae, cardiac signs and the failure to isolate bacteria from the blood stream after repeated attempts. It was believed that the most probable diagnosis was that of undulant fever since on four occasions there was complete agglutination of Brucella organisms in a dilution of 1 : 320, and a partial agglutination in a dilution of 1 : 640.

The clinical course of the disease was characterized by an undulating type of fever which subsided approximately six months after the onset of illness. The data in the case up to this time were typical of the ordinary form of undulant fever.

The complication in the course of undulant fever presented by this patient occurred after a period of about twelve weeks, during which time his temperature had remained normal. There was a sudden recurrence of the febrile state on exposure to sunlight, and the patient again became critically ill. A diagnosis of undulant fever was confirmed by the isolation of Br. melitensis from specimens of venous blood. During this relapse the patient had a sudden attack of severe, constant pain in the right upper quadrant which, along with other signs, was suggestive of acute cholecystitis. At operation the gallbladder was found to be infected, but of considerable additional interest was the finding of a granulomatous process in the liver.

The importance of these structures as a cause of the recurrence of the patient's illness is suggested by his freedom from symptoms relative to brucelliasis for a period of two years after their removal.

Pathologic Features.—The changes in tissues produced by Brucella organisms in animals are well known from studies [7] made on guinea-pigs experimentally infected with the disease. The organisms become focalized in the liver, spleen and lymph nodes and the cellular reactions about them proceed from those of an exudative inflammatory process to the formation of granulomatous nodules which contain a central necrotic mass. These nodules during the early stages are tubercle-like and consist of lymphocytes, epithelioid cells, an occasional giant cell and a wall made up of connective tissue fibrils. In man, structural changes in the liver, spleen and lymph nodes have been noted as the usual lesions in autopsy material by Gregersen and Lund,[8] Ebskov and Har-

7. Jaffé, R. H.: Ueber die experimentelle Infektion des Meerschweinchens mit dem Bacillus melitensis (Bruce) und dem Bac. abortus (Bang), Virchows Arch. f. path. Anat. **238**:119, 1922.

8. Gregersen, F., and Lund, T. M.: De patologisk-anatomiske førändringer ved febris undulans, Hospitalstid. **74**:349, 1931.

pøth,[9] Heiberg,[10] Wohlwill [11] and others. It is generally agreed that the nodules appearing in the human viscera are quite similar in their cellular components to those found in the lower animals. It seems apparent in the case reported here that the lesions in the parenchyma of the liver and the wall of the gallbladder were of such granulomatous character.

Final diagnosis of Br. melitensis as the etiologic factor responsible for these changes in the tissues was established by the growth and identification of these organisms through appropriate cultural and diagnostic methods.

The importance of these pathologic data becomes of greater clinical significance in the light of certain laboratory studies. Kennedy [12] recovered Br. melitensis in pure culture from the bile in two out of eight cases, while Eyre [13] stated that the micrococcus might be found in the bile in a "fair proportion of cases." These observations lead to the conclusion that, like typhoid fever, brucelliasis in man may be a more frequent predisposing factor in the development of cholecystitis and perhaps cholelithiasis than is generally supposed.

SUMMARY

1. A case of brucelliasis in man is reported. The diagnosis during the early course of the disease, although obscure, was suggested by the agglutination of specific antigens and was substantiated later during a relapse by the cultivation of Br. melitensis from specimens of venous blood.

2. The course of the illness was complicated by the occurrence of cholecystitis and focalized hepatitis. Disappearance of symptoms referable to active undulant fever occurred after the surgical removal of the infected tissue.

3. The local changes in the liver in this case are similar to those produced experimentally in the guinea-pig and rabbit.

4. Attention is called to the possible importance of undulant fever as a predisposing factor in the development of cholecystitis.

9. Ebskov, C., and Harpøth, H.: Et tilfaelde af febris undulans (Bang), Ugesk. f. laeger **37**:872, 1930.

10. Heiberg, S.: Nogle tilfaelde af febris undulans, Hospitalstid. **73**:933 (Sept. 18) 1930.

11. Wohlwill, F.: Zur pathologischen Anatomie der Bangerkrankung des Menschen, Virchows Arch. f. path. Anat. **286**:141, 1932.

12. Kennedy, J. C.: Report of the Commission for the Investigation of Mediterranean Fever, 1906, pt. 4, p. 93.

13. Eyre, J. W. H.: in von Kolle, W., and von Wassermann, A.: Handbuch der pathogenen Mikroorganismen, Jena, Gustav Fischer, 1912, vol. 4, p. 441.

RELATION OF PORTALS OF ENTRY TO SUBACUTE BACTERIAL ENDOCARDITIS

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As part of an investigation in subacute bacterial endocarditis the records of 364 patients admitted to Mount Sinai Hospital in the past fourteen years (to October 1933) have been analyzed, with particular attention to the antecedent history and the manner of onset. Several interesting facts were noted which are reported here.

The outstanding feature of the cases studied is the frequency with which the onset of subacute bacterial endocarditis is associated with an immediately preceding acute infection of the upper respiratory tract, tonsillitis or grip. This relationship has been noted by others. Libman,[1] in an article on the prognosis in subacute bacterial endocarditis, pointed out that the onset can occur with an attack of acute tonsillitis. He also wrote that "there is evidence which indicates that repetition of infection (in cases with recurrent attacks) may be due to an invasion from focal infections." In a subsequent article [2] he and his co-workers again drew attention to this fact. Oille, Graham and Detweiler,[3] in a discussion of the streptococcic bacteremia in endocarditis, pointed out that, in their opinion, endocarditis more frequently follows tonsillitis in children than is commonly believed.

There is also a good deal of bacteriologic and immunologic work which lends evidence to this conclusion. Kreidler,[4] in a study of the organisms isolated from 14 cases of subacute bacterial endocarditis (13 nonhemolytic streptococci and 1 beta hemolytic strain), pointed out the striking serologic individuality of each of the nonhemolytic organisms. There was no cross agglutination or complement fixation demonstrable, and immune bodies were present in the patients' serum against the homologous invader only. The fermentation reactions also varied.

From the medical service of Dr. B. S. Oppenheimer, the Mount Sinai Hospital.

1. Libman, E.: A Consideration of the Prognosis in Subacute Bacterial Endocarditis, Am. Heart J. **1**:25 (Oct.) 1925.

2. Rothchild, M. A.; Sacks, B., and Libman, E.: The Disturbances of the Cardiac Mechanism in Subacute Bacterial Endocarditis and Rheumatic Fever, Am. Heart J. **11**:356 (April) 1927.

3. Oille, John A.; Graham, Duncan, and Detweiler, H. K.: Streptococcus Bacteremia in Endocarditis, J. A. M. A. **65**:1159 (Oct. 2) 1915.

4. Kreidler, W. A.: Bacterial Endocarditis: Subacute, J. Infect. Dis. **39**:200, 1926.

The same bacteriologic and immunologic heterogenicity exists among the streptococci of the upper respiratory tract. In fact, this is probably the only location in the body in which such a flora is constantly present and which can furnish the necessary nidus and portal of entry for the establishment of an endocardial implantation. The work on the flora of the upper respiratory tract is too voluminous to be quoted in detail. Hooker and Anderson [5] studied 2,057 colony strains of streptococci from 101 specimens of sputum and reported the dominant aerobic strains to be of the nonhemolytic (alpha) type. Gamma types were also present, but rarely dominant. This parallels the observations on blood cultures in subacute bacterial endocarditis. They also showed the same bewildering heterogenicity among their nonhemolytic (alpha) streptococci that Kreidler noted in his blood cultures. Using cross-agglutination and complement fixation, they found less than 3 per cent which showed any antigenic relationship in 616 tests. No identical strains from different sources were discovered. Arnold [6] made two series of throat cultures, one three months before and one during an epidemic of influenza. In the first series 134 throats were investigated, of which 91 per cent were found to harbor nonhemolytic streptococci. In the group with influenza 116 cases were studied, and they all yielded, without exception, the nonhemolytic streptococcus.

A good deal of work in this field has been done on extirpated tonsils. Polvogt and Crowe,[7] in cultures from tonsils following 100 operations, found the alpha and beta types of streptococci in 91 per cent. Bartlett and Pratt [8] found streptococci present in 75 per cent of their cases, of which 22 per cent revealed the nonhemolytic variety. Kilduffe and Hersohn [9] studied 409 tonsils and found nonhemolytic streptococci in only 5 per cent. Julianelle,[10] in a similar study, found them in 31 per cent.

This accumulation of clinical and bacteriologic evidence, which can be multiplied, suggests with fair decisiveness that the upper respiratory passages harbor the foci which act as the most common portal of entry in subacute bacterial endocarditis. It also suggests the important con-

5. Hooker, S. B., and Anderson, L. M.: Heterogenicity of Streptococci Isolated from Sputum, J. Immunol. **16**:281, 1929.

6. Arnold, L.: Classification of Streptococci, J. Lab. & Clin. Med. **5**:587, 1920.

7. Polvogt, L. M., and Crowe, S. L.: Predominating Organisms Found in Cultures from Tonsils and Adenoids, J. A. M. A. **92**:962 (March 23) 1929.

8. Bartlett, F. H., and Pratt, J. S.: Streptococci Isolated from Excised Tonsils and Posttonsillectomy Blood Cultures, Am. J. Dis. Child. **41**:285 (Feb.) 1931.

9. Kilduffe, R. A., and Hersohn, W. W.: Cultures of Tonsils, J. Lab. & Clin. Med. **12**:788, 1927.

10. Julianelle, L. A.: Cultures of Tonsils, J. Lab. & Clin. Med. **9**:699, 1924.

clusion that transient bacteremia is not an infrequent occurrence in the apparently benign infections in this region.

There are other less common portals of entry. Horder,[11] in an early communication, wrote that the portal of entry may be not only through the mouth and throat but through the intestinal tract as well. Libman,[12] in discussing "operative bacteremia," the bacteremia established by an operative manipulation or surgical dressing, pointed to infections in the male and female genito-urinary tracts and to phlegmons and wounds as a possible portal of entry, and stated that a resulting endocarditis frequently occurs. Thayer[13] made the important observation that "many instances of endocarditis due to Streptococcus viridans owe their origin to pyorrhoea alveolaris or abscesses about the roots of the teeth." One of our patients gave a history of an immediate preceding infection of a hand, another of an otitic infection, one of a miscarriage, another of a cystoscopy and several of instrumental abortion. The latter cases and the inference that can be drawn from observations on gonorrheal endocarditis lead one to regard the genito-urinary tract as a not uncommon portal of entry for streptococcic endocarditis, probably only second in importance to the upper respiratory tract.

Another significant fact established by the study of our cases is the frequency with which a preexisting pathologic condition of the heart was demonstrated. In almost every case in which an adequate history was available an earlier abnormal cardiac condition was demonstrable. Libman[1] and his co-workers[2] drew attention to this and indicated that preexisting valvular or myocardial disease is a factor in subacute bacterial endocarditis which needs further study. Horder[11] obtained a history of preexisting rheumatic fever in 72 of his 150 cases. Of 118 postmortem examinations 80 per cent revealed preexisting valvular lesions on gross pathologic study. He also noted that a congenital defect predisposes the heart to infection. In the larger number of cases in my series the history was of specific rheumatic fever with cardiac involvement, chorea or typical rheumatic arthritis. In a smaller number atherosclerotic cardiac disease or a congenital anomaly was present. Syphilitic cardiac involvement can also be the precursor of subsequent subacute bacterial endocarditis. This is to be inferred from a case in the series (which came to necropsy), in which an acute endocarditis was engrafted on syphilitic aortitis. Though

11. Horder, Thomas: Infective Endocarditis, Quart. J. Med. **11**:319, 1908-1909.

12. Libman, E.: General Infections by Bacteria, J. Michigan M. Soc. **23**:462 (Nov.) 1924.

13. Thayer, W. S.: Bacterial (Infective) Endocarditis, Johns Hopkins Hosp. Rep. **22**:173, 1926.

exceptions undoubtedly may be found, it can be accepted as practically axiomatic that subacute bacterial endocarditis requires the fertile soil of an earlier endocarditis, rheumatic, atherosclerotic or syphilitic, or a congenital cardiac lesion on which to implant itself. Libman also included scarlet fever as one of the diseases which may furnish the necessary pathologic state of the heart.

Beside the history of infection of the upper respiratory tract in patients with earlier cardiac disease I have, in several cases, encountered a history of trauma in this region, such as tonsillectomy or extraction of a tooth, which closely antedated the onset of the symptoms. This points to the logical inference that these apparently minor surgical procedures may be accompanied by bacteremia, and in people with earlier cardiac disease result in an endocardial implant. Rushton [14] reported 4 cases of subacute bacterial endocarditis (from a series of 40 studied) in which onset dated from the extraction of a tooth. Bernstein [15] described the case of a young man of 25, with a history of chorea and rheumatic fever, in whom fever and the clinical picture of subacute bacterial endocarditis developed 5 days following the extraction of a tooth.

That surgical intervention in this and other parts of the body is frequently accompanied by bacteremia has been demonstrated. Schwarz and Frisch,[16] in discussing the negative results obtained by Rubin, Epstein and Werner,[17] reported blood cultures from 11 patients before, during and after tonsillectomy. All the cultures taken before and after operation were sterile. Cultures in 3 cases taken during the operation were positive. One of these showed a hemolytic streptococcus, another an anhemolytic streptococcus and the third a streptococcus which grew in one flask and died in subculture. Dabney [18] reported a streptococcic bacteremia two weeks following a tonsillectomy, the development of an acute infection of the mastoid process which on operation showed only staphylococci, the subsequent development of the picture of a lateral sinus thrombosis followed by negative findings at operation and the ultimate spontaneous recovery of the patient. He assumed that a local infected thrombus in the tonsillar fossa fed into the blood stream.

14. Rushton, M. A.: Subacute Bacterial Endocarditis Following Extraction of Teeth, Guys Hosp. Rep. **80**:39 (Jan.) 1930.

15. Bernstein, M.: Subacute Bacterial Endocarditis Following Extraction of Teeth, Ann. Int. Med. **5**:1138 (March) 1932.

16. Schwarz, H., and Frisch, J. A.: Blood Cultures After Tonsillectomy, Am. J. Dis. Child. **38**:1282 (Dec.) 1929.

17. Rubin, M. I.; Epstein, I. M., and Werner, Marie: Blood Cultures After Tonsillectomy, Am. J. Dis. Child. **38**:728 (Oct.) 1929.

18. Dabney, V.: Bacteremia Due to Tonsillectomy Complicated by Mastoiditis, Arch. Otolaryng. **2**:362 (Oct.) 1925.

Bacteremia incident to operation in other infected areas has been frequently reported. Seifert [19] reported 9 cases of 43 investigated in which bacteremia was demonstrated during appendectomy. He also obtained 60 per cent positive blood cultures during a series of operations on bone and 40 per cent during a series of operations on soft parts. Harter [20] pointed out that a positive blood culture following mastoidectomy is probably common and is not necessarily an indication of lateral sinus thrombosis. Schottmüller [21] demonstrated the presence of bacteremia during curettage. Libman [22] and Libman and Celler [23] described many cases of postoperative bacteremia and bacteremia after trauma.

The demonstration of bacteremia during operation in an infected area led me to determine how many patients in my series dated the onset of symptoms from some form of surgical intervention, particularly in the mouth and throat, such as tonsillectomy or extraction of a tooth. Ten gave such a history. These cases are reported here. In a number of others the history was too inadequate to warrant consideration. In many earlier cases the history of an antecedent operation was not inquired into. The incidence, therefore, of 10 cases in 364 studied is probably much lower than subsequent investigation may show.

The relevant facts in the cases in which an etiology of trauma in the upper respiratory passages is indicated are as follows:

REPORT OF CASES

Case 1.—A girl, aged 21, had chorea at the age of 12 and an attack of rheumatic fever at the age of 15. She was in bed for three months with the latter attack, and was informed that her heart was involved. At the age of 19 she suffered from another attack of rheumatic fever, and was hospitalized for three weeks. Following this she felt well for one and a half years, and was able to carry on fairly strenuous work.

Eighteen months before admission she had a sore throat, following which a tonsillectomy was performed. Since this operation the patient had been growing progressively more weak and tired; she had had transient pains in the legs and back, and her appetite had been poor. For two weeks before admission to the hospital she suffered from a persistent severe headache and for a week had been aware of fever.

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22. Libman, E.: On Some Experiences with Blood-Cultures in the Study of Bacterial Infections, Bull. Johns Hopkins Hosp. **17**:215 (July) 1906; The Value of Bacteriological Investigations in Otology with Special Reference to Blood Cultures, Arch. Otol. **37**:22 (Feb.) 1908.

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Physical examination revealed a girl who was obviously chronically ill. There was one petechia on the left shoulder and another in the left lower conjunctiva. Her heart was enlarged, and an accentuated first sound preceded by a rumbling crescendo presystolic murmur was present. This was followed by a loud blowing systolic and a soft diastolic murmur. The urine showed red blood cells on two occasions. The blood culture was positive for anhemolytic streptococci, with 200 colonies per cubic centimeter.

The patient was discharged unimproved.

CASE 2.—A woman, aged 30, could not give a satisfactory history, but knew of no specific cardiac disease. She said, however, that she had suffered from dyspnea and palpitation on moderate exertion, such as climbing one flight of stairs. She had had two therapeutic abortions.

Twelve weeks previous to admission she had a tooth extracted, immediately following which she noted general malaise, chilly sensations and fever. Then left nasal hemianopia developed and ten days following the onset of symptoms she had a chill. This was followed by several recurrences. She also had drenching sweats and arthralgia. She noted redness and tenderness of the finger-tips. During the three days before admission, she had two severe attacks of abdominal pain. On admission the patient showed pronounced anemia. There was a petechia in the right lower conjunctiva and a hemorrhage in the disk of the left fundus. The heart was enlarged, and presented the classic murmurs of advanced mitral stenosis and insufficiency. There was also present bilateral costovertebral tenderness, clubbing of the fingers and a café au lait complexion. The blood culture was positive for Streptococcus viridans, with 25 colonies per cubic centimeter.

The patient grew worse rapidly and died. A postmortem examination was not permitted.

CASE 3.—A boy, aged 15, four years previous to admission was examined by a doctor on the occasion of a gastric disturbance and was told that he had a cardiac lesion. Otherwise, the boy looked well and gave no evidence of cardiac disability. He had had tonsillitis two years before examination. The history was otherwise irrelevant.

Eight weeks following a tonsillectomy he noted progressive anemia, weakness, emaciation and loss of weight. He recovered sufficiently to return to school for a short period. He soon, however, had to go to bed, with a temperature of 102 F., general malaise, prostration and anorexia. Four weeks previous to admission he had had an attack of motor aphasia which lasted for one day. During the four weeks the renal function had diminished, and he passed only small amounts of highly colored urine. During the same period edema of the face, scrotum and lower extremities had been developing.

On admission he was found to be extremely ill, with marked anemia. He was lethargic and showed edema of the face, legs, scrotum and abdominal wall. There was one petechia present in the left lower eyelid. His heart was enlarged, and a loud double mitral murmur was present. The spleen and liver were palpable. Slight costovertebral tenderness was present and suggestive clubbing of the fingers. The urine contained red blood cells.

The blood culture showed anhemolytic streptococcic (anaerobic) bacteremia.

The patient died after four weeks in the hospital, and the clinical and bacteriologic findings were confirmed at autopsy.

The heart showed subacute bacterial endocarditis involving the mitral valve and the left auricle, with older healed lesions of the interventricular septum and aortic valves.

CASE 4.—A woman, aged 25, gave a history of having "caught cold" about seven years earlier, shortly after childbirth. One month later, arthralgia developed in the toes, the pain migrating upward to the ankles and then to the knees. There was no involvement of other joints, and she recalled no redness, swelling or fever. The pain persisted for about a year. Following this dyspnea developed, with attacks of palpitation on moderate exertion. She also had occasional sticking pains over the precordium.

Two months previous to admission she had a molar extracted. The following afternoon she noted chilly sensations, and that evening a fever developed. She had lost 9 pounds (4.1 Kg.) in four weeks, and was growing progressively weaker.

The physical examination showed the patient to be an anemic, underweight young woman. A precordial thrill, a sharp systolic impulse and the murmurs of mitral stenosis and insufficiency were present. The spleen was palpable. No embolic phenomena were noted.

The blood culture was positive for Str. viridans, with 18 colonies per cubic centimeter.

She had a febrile course, with a temperature up to 102 F., and died after one month in the hospital. Autopsy confirmed the clinical and bacteriologic observations. The mital valve was tightly stenosed, the cusps were densely fibrotic, and superimposed on the auricular aspect of both cusps there was an irregular, friable, grayish-white, fungoid, bacterial vegetation which was adherent to the leaflet and showed organization.

CASE 5.—A man, aged 31, had had acute rheumatic fever seven years previously, which left him with mitral stenosis and insufficiency and a possible aortic insufficiency.

He had been having some arthralgia, and was advised to have several teeth extracted. He followed this advice, and three weeks later noted the onset of fever and sweats. These had continued for four months, up to the time of admission to the hospital. Cachexia had been progressive. Three weeks before admission he noted some painful red spots on the volar aspect of the tips of several fingers, and he also felt a sudden sharp pain in the left upper quadrant of the abdomen.

On admission he appeared to be ill and emaciated. His skin was café au lait in color; a petechia was found in the left supraclavicular region and a Janeway node in the left hypothenar eminence. Marked sternal tenderness was present. The heart was enlarged downward and to the left. A rough systolic and a soft diastolic murmur were audible at the apex. He showed slight clubbing of the fingers, and the spleen was palpable. Red blood cells were found in the urine.

Two blood cultures were positive for Str. viridans, with 26 and 30 colonies per cubic centimeter, respectively.

He was discharged after five weeks, unimproved.

CASE 6.—A girl, aged 16, was discovered to have a congenital heart lesion at the age of 6. She had three previous admissions to the hospital, the first, seven years before for thrombocytopenic purpura. On her third admission, three years before the last admission, a splenectomy had been performed. She had been free from symptoms since.

Three months before admission the patient had an aching tooth extracted. Four weeks later she began to note fatigue, malaise, blurring of vision and headaches. At about the same time she suffered a sudden and severe abdominal cramp, localized just to the right of the umbilicus. She had a temperature of 100 F. and continued to have a fever up to the time of admission. For four days previous to admission she had a dull pain in the left thumb and in the great toe of the right foot.

The essential findings in the physical examination were a loud to-and-fro murmur, a heart enlarged to both the right and the left and sternal tenderness. Two blood cultures showed 150 and 130 colonies of Str. viridans per cubic centimeter, respectively.

She was discharged after hospitalization for seventeen days, her condition unchanged.

CASE 7.—An 8 year old girl, was admitted with a history of chorea, nine months prior to the present admission. The chorea lasted about six weeks, followed by a period of comparative well-being. Five months previously, following the extraction of two teeth she began to have a septic temperature. Associated with this were occasional vomiting and general malaise. Seven weeks before and again six weeks before she was given a transfusion of blood. She showed slight improvement for about two weeks. At this time another tooth was extracted, with a resultant flare-up of temperature to from 102 to 103 F. and an increase in her general malaise. Two weeks prior to admission she had two attacks of sharp cramplike pain in the epigastrium. She was reported to have had paralysis of all the extremities at this time, with a gradual clearing up of the condition in three days, only the left arm still showing some weakness. She had been growing progressively weaker, and was admitted for observation.

On admission she presented an acutely ill and septic appearance. There were petechiae on the neck and anterior wall of the chest; some dulness and râles were heard at the base of the right lung anteriorly. The heart was enlarged; a soft systolic murmur was present at the apex, and a harsh impurity of the first sound was heard in the second and third interspaces to the left of the sternum. The liver and spleen were palpable. The neurologic changes suggested left cerebral thrombosis. The blood culture was positive for Str. viridans.

The child's condition grew worse rapidly, and, she died thirty hours after admission. Postmortem examination was not permitted.

CASE 8.—A chauffeur, aged 33, had never had symptoms or signs suggestive of rheumatic fever, chorea or cardiac disease. His family history was irrelevant. He was referred to the hospital with a history of pain in the right lower quadrant of three days' duration, accompanied by a slight fever and marked abdominal tenderness. There had been no vomiting. Acute inflammation of the appendix was considered, and the patient was admitted to the medical service for observation.

Two and one-half months earlier he had a tonsillectomy performed. One week following this profuse sweats began and he noted considerable malaise. No other symptoms developed until two weeks before admission, when he began to have chills and a temperature. Herpes labialis appeared one week before admission.

It was noted on admission that beside the tenderness in the right lower quadrant the spleen was easily palpable and that a rough systolic murmur was audible. Red blood cells were present in the urine. The blood culture showed Str. viridans bacteremia.

During hospitalization of eight weeks the patient presented numerous embolic phenomena, which included renal infarction, petechiae, lesions of the fundus and an embolus into the right axillary artery, for which an exploratory embolectomy was attempted. The patient died following signs of mesenteric thrombosis.

Autopsy revealed no evidence of rheumatic cardiovalvular disease. There was present, however, a congenital lesion of the aortic valve (a bicuspid aortic valve), with a rudimentary third leaflet, on which the vegetations of the subacute bacterial endocarditis were engrafted. The valve leaflet had been perforated.

Case 9.—A man, aged 24, with cardiac disease had been visiting the cardiac clinic for many years. Two years previous to the present admission he had been hospitalized for an attack of acute rheumatic fever with arthritis and further cardiac involvement.

Eleven weeks before admission he had a tooth treated and then extracted. An apical abscess was found. Two weeks following the incident he noted general weakness, malaise and increasing pallor. His condition became progressively worse, and a record of his temperature showed daily rises, on some days up to 101 F. Five weeks before admission a pain developed in the left leg which persisted for six or seven days. One week later a similar pain appeared in the right arm and shoulder, with inability to raise the arm. This also cleared up. Ten days before admission arthralgia developed in the right toe and in the ring fingers of both hands, and four days later a sharp sticking pain appeared in the left upper quadrant of the abdomen. Just before admission he noted orthopnea and nocturnal dyspnea.

Physical examination revealed the patient to be acutely ill, dyspneic and orthopneic, and his temperature 101 F. A petechia was present in the left lower conjunctiva. The heart was enlarged to the right and left, and the murmurs and dynamics of mitral and aortic insufficiency and aortic stenosis were present. There was tenderness in the left upper quadrant of the abdomen. Clubbing of the fingers and hematuria were noted.

The blood culture was positive for Str. viridans, with 35 colonies per cubic centimeter. The patient died five weeks after admission. An autopsy was not permitted.

Case 10.—A married woman, aged 33, a quadripara, had had one stillbirth, one spontaneous abortion at ten weeks and two normal deliveries. Eighteen years before admission she had an attack of "tonsillitis," followed by stiffness of both hands and wrists and then of the shoulders. This lasted for three months and recurred twice during the two succeeding years, each attack lasting approximately the same length of time. Eleven years before admission she was told that she had a cardiac murmur. For the preceding eleven years she had had occasional sharp pains to the left of the sternum which came on suddenly and lasted only a few minutes. One year before admission she suffered from an attack of palpitation, with a regular cardiac rate of 150. At this time she was in bed for two weeks. Four weeks following the first attack she had a second. Rest in bed for one day corrected this.

The more immediate history dates back six months previous to admission, when she began to have pain on bending the metacarpal phalangeal joint of the right index finger. This persisted to the time of admission to the hospital. One month before hospitalization she had pain in the left mandibular joint, which lasted for a few days and recurred two weeks later. This was still present at the time of admission, and made chewing painful.

Ten weeks before admission she had all her upper teeth and one of her lower teeth removed. Since then she had lost 8 pounds (3.6 Kg.), and for the past six or seven weeks her temperature had been from 100 to 102 F. during the day, rising higher during the night. She had a shaking chill lasting from ten to fifteen minutes, seven days before admission. There had been no embolic manifestation and no syncope.

Physical examination revealed the patient to be well developed and well nourished. The upper jaw was edentulous, and only six teeth remained in the lower jaw. The eyes, ears, nose, throat and lungs were essentially normal. The heart was enlarged downward and to the left to the anterior axillary line. There

was prominence of the pulmonary conus. The second pulmonic sound was louder than the aortic. There was a presystolic thrill and a crescendo murmur at the apex. A low systolic murmur was present at the base.

The liver was palpable about 1 cm. below the costal margin, and the spleen could just be felt.

The patient was in the hospital for seven and a half weeks during her first admission. At that time the temperature ranged between 100 and 102 F.; occasionally it rose to 103 or 104 F.

The sedimentation time was eighteen minutes. The white blood cell count was 10,000, with 64 per cent polymorphonuclears. The hemoglobin content was 76 per cent. The urine occasionally showed some erythrocytes. Three blood cultures were positive for the alpha streptococcus.

While she was under observation there developed an Osler node on the left great toe, a splenic infarct and clubbing of the fingers. The hemoglobin content dropped to 58 per cent at the time of her discharge.

She was readmitted moribund four months later and died within five minutes. An autopsy was not permitted.

The sequence of events in this case was probably implantation on the endocardium resulting from the dental trauma in a person with rheumatic cardiac disease at a time when she was having a recurrent attack of low grade rheumatic fever.

SUMMARY

Results of a study of 364 cases of subacute bacterial endocarditis suggest that the common portal of entry of the streptococcus is from foci of infection in the passages of the upper respiratory tract and the mouth. Other portals of entry are the genito-urinary tract, otitic infections and wound infections. I have found no evidence of the gastro-intestinal tract as the portal of entry in any of the cases. The onset with an acute infection of the upper respiratory tract or tonsillitis in many cases led to the conclusion that in these apparently benign infections a transient bacteremia may occur. The frequency of antecedent cardiac disease, usually rheumatic but sometimes atherosclerotic or syphilitic, or some congenital lesion, indicated that the streptococcus probably requires a previously diseased or abnormal endocardium on which to implant itself.

Ten cases of subacute bacterial endocarditis are reported, in which a history of tonsillectomy or extraction of a tooth closely antedated the onset of symptoms, indicating that trauma of an infective focus about the teeth and pharynx may produce transient bacteremia and thus play a part in promoting implantation on the endocardium.

EXPERIMENTAL RENAL INSUFFICIENCY PRODUCED BY PARTIAL NEPHRECTOMY

III. DIETS CONTAINING WHOLE DRIED LIVER, LIVER RESIDUE AND LIVER EXTRACT

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Chanutin and Ferris [1] reduced renal tissue in the rat to a minimum compatible with life and demonstrated a progressive development of polyuria, albuminuria, retention of nitrogen, renal hypertrophy, hypertension and cardiac hypertrophy. The animals were fed a stock diet containing a moderate amount of protein. Wood and Ethridge [2] reported pathologic changes in the kidney stumps of partially nephrectomized rats. They noted changes in the glomeruli, tubules, small arteries and arterioles. The arterial lesions were similar to those noted in human arteriosclerosis. In the physiologic and pathologic changes that are seen after subtotal nephrectomy an excellent opportunity is offered for studying the influence of various combinations of foodstuffs and metabolites on the damaged kidney.

The present study was undertaken to ascertain the effect of feeding diets containing whole liver, liver protein and a concentrated aqueous extract of liver to animals with acute and potential renal insufficiency. The degree of renal insufficiency, hypertension and azotemia and the relationship of the size of the heart, kidney and liver to the surface area were studied by the methods described by Chanutin and Ferris.[1]

The effect of diets high in protein on the kidneys of experimental animals has been studied by numerous investigators. The literature has been reviewed by Mitchell,[3] Bischoff [4] and others. It is difficult to interpret the results reported, since there were variations in the diets, species of animals, the type and the amount of protein fed, the length of feeding time and the age of the animals. Estimates of the degree of damage to the kidney have been based almost entirely on histopathologic studies, though stress has also been laid on the number of casts in the urine,

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From the Laboratory of Physiological Chemistry, the University of Virginia.

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2. Wood, J. E., and Ethridge, C.: Proc. Soc. Exper. Biol. & Med. **30**:1039, 1933.

3. Mitchell, H. H.: J. Nutrition **1**:271, 1929.

4. Bischoff, Fritz: J. Nutrition **5**:431, 1932.

albuminuria and changes in the concentration of the nonprotein nitrogenous constituents of the blood.

The feeding of diets rich in liver to white rats has consistently produced renal damage. Newburgh and Curtis [5] and Newburgh and Johnston [6] demonstrated severe renal lesions in the white rat after feeding them diets containing 40 per cent or more whole dried liver. From their experiments, they believed liver to be the most nephrotoxic of the natural foods studied. Blatherwick and his co-workers [7] demonstrated marked renal changes in the kidney of unilaterally nephrectomized rats fed a diet rich in whole dried liver. Both Mitchell [3] and Newburgh and Johnston [6] postulated that the renal damage in these cases might have been due to substances present in natural foods other than the protein. The latter workers showed that small quantities of sodium nucleate were capable of producing renal lesions.

The experiments reported in this paper demonstrate that the quantity of whole liver and the type of liver fraction may influence the syndrome noted in partially nephrectomized rats.

EXPERIMENTAL METHODS

The animals were raised in this laboratory. They were maintained on a prepared adequate stock diet until they were between 60 and 70 days old, when subtotal nephrectomy, unilateral nephrectomy or control laparotomy was performed. They were immediately given the experimental diets. A maximum number of six rats were kept in a cage and allowed food and water ad libitum up to the time of death. All animals were killed while in good health at definite intervals, with the exception of a few animals that appeared to be slightly ill, or acutely ill and about to die. No quantitative record of intake of food was attempted.

The details of the experimental procedure have been presented in a previous article,[1] but a brief summary of the methods used will be given here. All animals selected for the experiment were anesthetized with ether for the operation. Subtotal nephrectomy was performed by bringing the left kidney to the surface, placing loops of white cotton thread tightly in position around each pole and then ligating the renal tissue by closing the loops. By this method the circulation of the blood was shut off from about two thirds to three fourths of the left kidney. One week later the right kidney was excised. Unilateral nephrectomy was preceded by bringing the left kidney to the surface, exposing and then replacing it; the right kidney was removed one week later. The control animals underwent the same operative procedure, the kidneys being exposed, handled and replaced.

Twenty-four hour specimens of urine were obtained for the concentration test by placing the rats in individual metabolism cages. Food was withdrawn twenty-four hours before the rat was placed in the metabolism cage in order to eliminate the possible effect of food metabolites on the urinary excretion. Food and water

5. Newburgh, L. H., and Curtis, A. C.: Production of Renal Injury in the White Rabbit by the Protein Diet, Arch. Int. Med. **42**:801 (Dec.) 1928.

6. Newburgh, L. H., and Johnston, M. W.: J. Clin. Investigation **10**:153, 1931.

7. Blatherwick, N. R.; Medlar, E. M.; Connolly, J. M., and Bradshaw, P. J.: J. Biol. Chem. **92**:lxxxiv, 1931.

were withdrawn during the period of the concentration test. In a few cases, indicated in the tables, the concentration test was carried out without the preliminary removal of food. In the estimation of urinary protein heat was applied to cause coagulation, and the coagulum was washed, dried and weighed. All blood pressure readings were obtained by inserting a cannula in the carotid artery. Before inserting the needle the animals were anesthetized with ether. The final blood pressure reading was made while the reflexes were active, but before the animal struggled. Small quantities of blood necessary for the analysis of hemoglobin and nonprotein nitrogen were obtained by clipping the tail. The rat was exsanguinated by cutting the carotid artery. The atria of the heart were removed before weighing, so as to obtain the weight of the ventricles. The kidneys and liver were blotted free from excess blood and weighed immediately after removal. The surface area was calculated from the weight of the intact animal by the formula of Lee.[8]

Eight experimental diets were used. The composition of the diets is shown in table 1. The first six diets differed only in the percental concentration of the whole dried liver, which was the chief source of protein. Hog liver kept in cold

TABLE 1.—*Composition of Rations*

	Concentration of Component in Diet, per Cent									
Diet	Dried Whole Liver	Liver Residue	Liver Extract*	Meat Residue	Starch	Lard	Cod Liver Oil	Dried Yeast	Salt Mixture†	Nitrogen, per Cent
L. 10	10	..	..	..	62	14	5	5	4	1.4
L. 15	15	..	..	..	53	18	5	5	4	1.8
L. 20	20	..	..	..	52	14	5	5	4	2.2
L. 40	40	..	..	..	30	16	5	5	4	4.4
L. 60	60	..	..	..	12	14	5	5	4	6.2
L. 80	80	..	..	..	..	6	5	5	4	8.2
L. Res	..	80	..	..	..	6	5	5	4	9.5
L. Ext	..	..	30	20	37	..	5	5	4	3.9

* Expressed in cubic centimeters.
† From Osborne and Mendel (J. Biol. Chem. **37**: 572, 1919).

storage was purchased in large quantities. It was ground without the loss of tissue juice, dried in a large steam-heated container and ground to a fine powder. The protein in the seventh diet consisted of the residue of the liver after the water-soluble constituents had been removed. The eighth diet consisted of extracted beef muscle (meat residue) plus a concentrated solution of the water-soluble constituents of hog liver, 1 cc. of the extract representing 7.5 Gm. of whole liver.

For convenience, numerous abbreviations are used in this article. The first six diets are designated L. 10, L. 15, seriatim, in accordance with the percentage of the whole dried liver in the diet; the seventh diet, in which liver residue was used, is indicated by L. Res., and the eighth diet, in which meat residue plus aqueous liver extract was used, is indicated by L. Ext. The ratios of the weights of the heart, kidneys and liver to the surface area are expressed by

$\frac{H.W.}{S.A.}$, $\frac{K.W.}{S.A.}$ and $\frac{L.W.}{S.A.}$ respectively.

A detailed histopathologic study of the kidneys of the killed animals was made by K'ang.[9]

8. Lee, M. O.: Am. J. Physiol. **89**:24, 1929.
9. K'ang, H. J.: Unpublished data.

EXPERIMENTAL RESULTS

Controls.—Observations on the intact and on the unilaterally nephrectomized rats are shown in table 2. The urinary volume obtained in

TABLE 2.—*Normal Values in Control Animals Fed a Variety of Diets*

Rats	Renal Condition and Diet	Value	Weight, Gm.	Duration of Experiment, Days	Heart Weight / Surface Area ×100	Kidney Weight / Surface Area ×100	Liver Weight / Surface Area ×100	Blood Pressure, Mm.	Nonprotein Nitrogen, Mg. per 100 Cc.	Twenty-Four Hour Urine Concentration Test: Volume, Cc.	Twenty-Four Hour Urine Concentration Test: Specific Gravity
8	2 kidneys (L. 10)	Minimum	183	35	0.170	0.255	1.97	110	30	0.8	1.0332
		Maximum	262	241	0.180	0.413	2.47	132	52	2.8	1.0629
		Average			0.175	0.369	2.26	121	38	1.4	1.0495
7	1 kidney (L. 10)	Minimum	210	28	0.168	0.230	1.94	120	26	0.6	1.0395
		Maximum	336	219	0.206	0.330	2.50	140	64	3.1	1.0571
		Average			0.186	0.273	2.30	127	51	2.0	1.0471
5	2 kidneys (L. 15)	Minimum	194	50	0.160	0.357	2.14	112	32	0.2	1.0299
		Maximum	312	230	0.185	0.435	2.82	130	55	4.8	1.0674
		Average			0.177	0.410	2.47	120	45	2.3	1.0466
11	1 kidney (L. 15)	Minimum	176	50	0.166	0.241	1.94	114	29	0.6	1.0289
		Maximum	404	289	0.195	0.438	2.61	152	51	5.0	1.0803
		Average			0.178	0.296	2.22	131	41	2.3	1.0522
13	2 kidneys (L. 20)	Minimum	167	56	0.170	0.350	1.98	108	27	1.0	1.0333
		Maximum	395	219	0.205	0.496	3.25	154	57	4.7	1.0747
		Average			0.188	0.414	2.55	121	40	2.9	1.0532
10	1 kidney (L. 20)	Minimum	174	14	0.156	0.233	1.85	106	27	0.7	1.0338
		Maximum	362	254	0.199	0.344	3.46	130	56	4.6	1.0678
		Average			0.175	0.290	2.52	119	39	2.2	1.0544
13	2 kidneys (L. 40)	Minimum	216	47	0.168	0.423	2.62	112	32	1.5	1.0331
		Maximum	385	202	0.208	0.567	3.90	150	55	4.1	1.0740
		Average			0.185	0.491	3.10	131	39	3.1	1.0524
9	1 kidney (L. 40)	Minimum	209	14	0.167	0.315	2.34	110	32	1.3	1.0437
		Maximum	364	92	0.205	0.398	3.76	148	45	5.9	1.0778
		Average			0.181	0.344	2.81	123	39	2.7	1.0594
9	2 kidneys (L. 60)	Minimum	178	23	0.156	0.417	2.58	110	28	0.9	1.0386
		Maximum	387	185	0.219	0.548	3.48	150	53	3.7	1.0666
		Average				0.185	3.02	127	38	2.4	1.0522
6	1 kidney (L. 60)	Minimum	207	15	0.171	0.345	2.58	114	29	0.7	1.0405
		Maximum	332	133	0.192	0.374	3.26	146	48	6.3	1.0734
		Average			0.184	0.359	2.94	129	42	3.5	1.0559
18	2 kidneys (L. 80)	Minimum	155	27	0.154	0.447	2.89	106	34	0.6	1.0388
		Maximum	352	331	0.216	0.702	3.82	142	75	4.7	1.0725
		Average			0.181	0.577	3.26	126	53	2.6	1.0561
18	1 kidney (L. 80)	Minimum	150	14	0.137	0.316	2.57	108	35	2.0	1.0347
		Maximum	360	204	0.212	0.525	4.16	160	85	8.9	1.0559
		Average			0.180	0.456	3.27	125	47	4.5	1.0412
11	2 kidneys (L. Ext. 80)	Minimum	150	73	0.155	0.370	2.55	104	38	1.8	1.0387
		Maximum	346	226	0.206	0.765	3.75	132	97	4.2	1.0789
		Average			0.182	0.588	2.95	118	64	2.6	1.0549
17	1 kidney (L. Ext. 80)	Minimum	127	30	0.141	0.328	1.81	108	34	1.7	1.0343
		Maximum	361	226	0.202	0.632	3.46	144	122	4.7	1.0664
		Average			0.169	0.475	2.80	120	74	3.1	1.0454
8	2 kidneys (L. Ext.)	Minimum	173	15	0.149	0.445	1.97	110	31	0.6	1.0350
		Maximum	310	176	0.216	0.583	3.02	124	49	3.5	1.0400
		Average			0.179	0.506	2.54	118	41	1.9	1.0563
4	1 kidney (L. Ext.)	Minimum	147	15	0.148	0.336	1.95	108	40	1.7	1.0367
		Maximum	315	176	0.178	0.365	2.58	126	46	3.0	1.0595
		Average			0.166	0.356	2.28	120	42	2.3	1.0479

the concentration test exceeded 5 cc. in only four of the one hundred and sixty-five controls. Although those animals receiving the 10 per cent whole liver diet had an unusually small urinary output, it may be said that in general the urinary volume of the entire series did not vary greatly with the dietary changes. The specific gravity of the urine varied considerably in individual members of the different groups, but

the average specific gravity in any one group was independent of the diet. The group averages fell between 1.0450 and 1.0594. Hence, it will be seen that the volume and the specific gravity of the urine excreted by the intact and unilaterally nephrectomized rats are not appreciably affected by the nephrectomy or the diet.

In a few cases in which a quantitative analysis for urinary protein was carried out, 0.05 Gm. was the maximum found.

Of the eighty-five intact control animals, there were three rats with blood pressure above 140 mm. of mercury. In the case of eighty unilaterally nephrectomized rats, eight animals had elevated blood pressure. The animals with hypertension were uniformly distributed among the various dietary groups. Unilateral nephrectomy apparently increased the tendency toward higher blood pressure.

The average value of the $\frac{\text{H.W.}}{\text{S.A.}}$ ratio did not change in the controls fed the different diets. Except in younger animals, there was a high degree of correlation between the $\frac{\text{H.W.}}{\text{S.A.}}$ ratio and the blood pressure reading.

The average values of the $\frac{\text{L.W.}}{\text{S.A.}}$ ratio were increased in the rats receiving the larger amounts of whole liver in their diets. Those animals on diets that were 40, 60 and 80 per cent whole liver presented definitely fatty livers. Animals receiving the diet containing liver residue had hypertrophy of the liver, but it was not so great as that seen in rats ingesting the diet that was 80 per cent whole liver. The livers of the rats on the diet containing liver residue were not fatty. There was no noticeable change in the size of the livers of the animals receiving the diet containing liver extract.

The value of the $\frac{\text{K.W.}}{\text{S.A.}}$ ratio was roughly proportional to the amount of total nitrogen present in the diets based on whole dried liver and liver residue. The increase in value of the average weight ratio of the kidneys of the intact control animals receiving 80 per cent whole liver was 56 per cent (0.577) greater than that of controls receiving 10 per cent whole liver (0.369). The liver residue caused a still greater hypertrophy (0.588), owing perhaps, to the greater concentration of total nitrogen. The kidneys of the animals fed the diet rich in liver extract were slightly larger than those of animals fed a diet based on whole liver containing a comparable amount of nitrogen. The degree of renal hypertrophy in the unilaterally nephrectomized rats was greater than that in the intact rats. In fact, the $\frac{\text{K.W.}}{\text{S.A.}}$ ratio of the group receiving the diet that was 80 per cent whole liver had a value 67 per cent (0.456) greater than that of the group receiving 10 per cent (0.273). The diet containing liver residue was responsible for the greatest hypertrophy (0.475). The renal hypertrophy of the rats fed the liver extract was about equal to that of the rats fed a diet that was 60 per cent whole liver.

Effect of Diet on Experimental Animals.—Observations on the partially nephrectomized rats are shown in tables 3 to 10.

Because the urinary volume, urinary specific gravity, urinary protein and blood pressure were within fairly fixed limits in all the control animals and did not vary appreciably with the different diets, the following values have been arbitrarily taken as the normal ranges for all animals on which experiments were done: urinary volume, from 0 to

TABLE 3.—*Observations on Partially Nephrectomized Animals Receiving 10 per Cent Whole Liver*

Rat	Weight, Gm.	Duration of Experiment, Days	Heart Weight / Surface Area ×100	Kidney Weight / Surface Area ×100	Liver Weight / Surface Area ×100	Blood Pressure, Mm.	Nonprotein Nitrogen, Mg. per 100 Cc.	Twenty-Four Hour Urine Concentration Test: Volume Cc.	Specific Gravity	Albumin, Gm.
1	151	28	0.169	0.174	1.74	130	32	5.0	1.0242	0.006
2	157	28	0.182	0.162	1.93	112	60	2.5	1.0294	.....
3	170	28	0.183	0.173	1.89	112	48	4.2	1.0229	.....
4	248	35	0.195	0.156	2.22	122	88	5.1	1.0320	.....
5	181	65	0.174	0.131	1.86	130	30	4.9	1.0386	.....
6	155	65	0.194	0.139	1.83	144	53	6.7	1.0204	0.012
7	180	77	0.158	0.170	2.08	116	28	1.8	1.0486	.....
8	170	77	0.166	0.173	1.79	132	..	2.5	1.0364	.....
9	192	77	0.176	0.191	1.95	120	34	2.5	1.0371	.....
10	174	118	0.177	0.092	2.05	106	42	2.1	1.0410	.....
11	170	118	0.188	0.157	1.98	124	38	2.5	1.0410	.....
12	205	118	0.229	0.231	2.13	142	46	3.2	1.0352	.....
13	208	118	0.205	0.225	2.24	144	32	2.7	1.0435	.....
14	163	142	0.168	0.174	2.05	120	40	2.5	1.0367	.....
15	140	142	0.171	0.162	2.20	120	44	1.3	1.0502	.....
16	150	142	0.180	0.131	1.91	144	45	4.0	1.0243	.....
17	202	142	0.192	0.193	1.96	146	48	4.6	1.0322	.....
18	165	142	0.187	0.177	2.22	106	47	2.1	1.0398	.....
19	171	142	0.170	0.265	2.04	118	44	0.8	1.0696	.....
20	104	152*	0.242	0.129	1.60	158	60	4.8	1.0219	0.023
21	96	152*	0.216	0.067	1.54	168	100	4.0	1.0229	.....
22	116	156*	0.242	0.164	1.79	160	..	3.6	1.0262	.....
23	172	161	0.177	0.116	1.94	122	46	2.1	1.0275	.....
24	200	161	0.184	0.197	1.71	134	..	5.8	1.0268	0.006
25	198	164	0.197	0.193	2.44	130	..	3.7	1.0290	.....
26	142	164	0.227	0.160	1.97	202	..	5.0	1.0251	0.034
27	168	164	0.252	0.164	1.90	206	74	9.5	1.0141	0.020
28	266	166	0.182	0.145	2.34	120	70	4.2	1.0318	.....
29	215	166	0.193	0.145	2.37	148	..	4.4	1.0264	.....
30	143	171	0.256	0.191	....	164	76	4.7	1.0232	.....
31	188	171	0.177	0.154	2.18	126	56	1.7	1.0471	.....
32	162	171	0.197	0.160	2.15	130	62	3.4	1.0360	.....
33	276	171	0.163	0.165	2.36	118	70	4.4	1.0264	.....
34	262	219	0.213	0.194	2.06	152	44	3.5	1.0345	.....
35	166	219	0.197	0.173	1.88	132	40	2.2	1.0297	.....
36	132	241	0.267	0.234	2.21	168	51	6.5	1.0228	0.000
37	128	241	0.257	0.254	1.70	162	59	7.9	1.0135	0.019

* Acutely ill.

5 cc.; urinary specific gravity, above 1.0400; urinary protein, from 0.000 to 0.05 Gm. and blood pressure, from 100 to 140 mm. of mercury. Because the nonprotein nitrogen of the control animals was influenced by the diet, the normal values for the experimental animals were determined by reference to the comparable control group. The heart was considered enlarged when the value of the $\frac{\text{H.W.}}{\text{S.A.}}$ ratio was greater than 0.2.

The specific differences apparently due to the different experimental diets are given in detail.

Ten Per Cent Whole Liver (Table 3): The urinary volume was within the normal range in 85 per cent of the animals; 16 per cent excreted between 5 and 10 cc. Despite the small volume of the urine, the specific gravity was low. The largest group (46 per cent) secreted urine with specific gravity between 1.0200 and 1.0300, and 30 per cent between 1.0300 and 1.0400. Only two rats secreted urine with specific gravity below 1.0200. The amount of protein excreted was determined in only eight animals, and the highest value was 0.034 Gm.

Normal blood pressure was seen in 60 per cent of the animals (thirty-seven). Five had blood pressure between 140 and 150 mm. up to the one hundred and fifty-second day after operation. At this time, the first readings of definitely increased blood pressure were seen in animals acutely ill (158, 168 and 160 mm.). After this period, six of the fifteen rats in good condition had blood pressures above 150, two readings being 202 and 206.

Normal values for nonprotein nitrogen (from 25 to 60 mg. per hundred cubic centimeters) were obtained in 74 per cent of the animals. Elevated values were seen during the latter part of the experiment. The highest concentration (100 mg.) was seen in an animal acutely ill.

The $\frac{\text{H.W.}}{\text{S.A.}}$ ratio could be correlated with the blood pressure only after the hundred and fifty-second day. The values of the $\frac{\text{K.W.}}{\text{S.A.}}$ ratio varied from 0.067 to 0.254. The average value (0.169) was representative of the majority of the kidney stumps. The values of the $\frac{\text{L.W.}}{\text{S.A.}}$ ratio varied from 1.54 to 2.44 (average, 2.0).

One of the outstanding findings in the study was the long time which elapsed before hypertension developed in these rats. Unquestioned hypertension was first observed about one hundred and fifty days after operation. The volume of urine excreted during the concentration test was small, and the specific gravity was below the value obtained in control animals excreting the same quantity of urine. The nonprotein nitrogen of the blood was variable in amount, but in the majority of cases the value was equivalent to that found in the control animals. The fact that hypertension developed slowly and preceded evidence of renal damage must be considered as an important finding.

Fifteen Per Cent Whole Liver (Table 4): The volume of urine excreted was within the normal range (from 0 to 5 cc.) in 48 per cent of the animals. Slightly increased amounts (from 5 to 8 cc.) were secreted by 43 per cent. Only three of the fifty-eight rats secreted more than 8 cc., the highest value being 11.6 cc. For the majority of the rats (52 per cent) the specific gravity was between 1.0200 and 1.0300; for 14 per cent between 1.0100 and 1.0200, and for 19 per cent between 1.0300 and 1.0400. The specific gravity seemed to be related roughly to the volume of urine. The protein excreted in the urine varied between 0.002 and 0.112 Gm.; 50 per cent of the values were between 0.000 and 0.050 Gm., and 50 per cent were above this range.

Normal blood pressure was seen in 52 per cent of the animals (sixty-four). Of those killed between the forty-eighth and fifty-eighth days after operation, seven of seventeen had blood pressure above 140 mm., the highest value being 192 mm. Between the hundred and fifteenth and two hundred and eighty-ninth days, four of the forty-seven had hypertension. It was possible to group the

rats with hypertension as follows: from 140 to 150, 14 per cent; from 150 to 160, 12 per cent; from 160 to 180, 17 per cent, and three had a pressure of 186, 190 and 192 mm., respectively.

TABLE 4.—*Observations on Partially Nephrectomized Animals Receiving 15 per Cent Whole Liver*

Rat	Weight, Gm.	Duration of Experiment, Days	Heart Weight Surface Area ×100	Kidney Weight Surface Area ×100	Liver Weight Surface Area ×100	Blood Pressure, Mm.	Nonprotein Nitrogen, Mg. per 100 Cc.	Twenty-Four Hour Urine Concentration Test: Volume Cc.	Specific Gravity	Albumin, Gm.
1	164	48	0.202	0.179	2.15	156	51	3.7	1.0236	.....
2	282	48	0.201	0.224	2.49	130	48	3.4	1.0450	.....
3	172	48	0.208	0.171	1.95	150	60	3.2	1.0351	.....
4	184	48	0.192	0.156	2.25	138	57	4.2	1.0323	.....
5	104	50	0.168	0.123	1.44	178	63	3.8	1.0225	.....
6	200	50	0.184	0.173	2.20	126	57	4.9	1.0261	.....
7	258	50	0.216	0.199	2.23	162	53	5.1	1.0308	.....
8	156	50	0.175	0.185	1.92	124	38	1.3	1.0420	.....
9	144	50	0.218	0.197	1.59	192	..	5.8	1.0211	.....
10	190	58	0.183	0.159	2.16	124	59	5.3	1.0254	.....
11	154	58	0.174	0.178	2.00	160	54	2.1	1.0449	.....
12	154	58	0.172	0.175	1.99	148	63	4.0	1.0244	.....
13	232	58	0.187	0.168	2.23	134	48	3.0	1.0416	.....
14	180	58	0.222	0.216	2.45	136	44	7.5	1.0192	.....
15	152	58	0.183	0.160	1.85	132	56	7.2	1.0190	.....
16	218	58	0.172	0.163	2.30	130	48	3.1	1.0401	.....
17	156	58	0.177	0.138	2.15	122	45	3.0	1.0304	.....
18	162	115	0.261	0.249	2.20	144	92	...	.....	.....
19	118	115*	0.247	0.230	1.64	154	108	...	.....	.....
20	150	139	0.161	0.152	1.55	114	52	...	.....	.....
21	252	143	0.181	0.219	2.38	120	67	...	.....	.....
22	228	143	0.223	0.177	2.17	144	83	7.4	1.0239	0.102
23	192	143	0.178	0.190	2.05	118	47	1.6	1.0500	.....
24	146	148	0.185	0.160	1.89	134	53	9.6	1.0141	0.006
25	182	148	0.178	0.148	1.63	102	43	7.9	1.0239	0.002
26	228	148	0.172	0.167	2.12	132	31	6.9	1.0288	0.016
27	266	148	0.182	0.194	1.85	136	39	3.5	1.0397	.....
28	162	154	0.216	0.179	2.08	160	40	6.8	1.0232	0.056
29	168	154	0.211	0.201	1.64	156	48	5.3	1.0237	0.030
30	184	154	0.194	0.177	2.18	126	46	3.2	1.0315	.....
31	364	158	0.192	0.197	2.38	122	39	3.5	1.0414	.....
32	218	158	0.210	0.264	2.42	154	57	7.5	1.0260	0.079
33	312	158	0.200	0.206	2.21	134	..	6.2	1.0291	0.048
34	122	160	0.233	0.128	1.58	160	66	7.5	1.0150	0.010
35	224	160	0.227	0.253	1.88	134	45	7.0	1.0220	0.05b
36	148	173	0.189	0.244	1.88	170	72	7.5	1.0220	0.065
37	144	173†	0.193	0.164	2.00	150	42	...	.....	.....
38	150	173*	0.231	0.242	1.94	162	..	...	.....	.....
39	210	180	0.192	0.174	2.10	170	55	2.1	1.0419	.....
40	178	180	0.205	0.216	2.05	174	55	4.3	1.0375	.....
41	244	180	0.163	0.157	2.02	124	50	5.8	1.0221	0.009
42	250	180	0.206	0.191	2.37	138	56	4.3	1.0305	.....
43	158	180	0.153	0.134	1.87	118	52	2.3	1.0280	.....
44	226	180	0.167	0.186	2.10	124	..	2.2	1.0228	.....
45	270	188	0.205	0.177	2.13	140	59	2.9	1.0419	.....
46	200	193	0.237	0.195	2.04	174	63	11.6	1.0191	0.050
47	238	193	0.189	0.199	2.00	140	48	3.7	1.0305	.....
48	146	198*	0.270	0.258	2.05	172	70	7.0	1.0205	0.047
49	274	198	0.189	0.245	2.01	136	..	4.2	1.0256	.....
50	230	198	0.231	0.199	2.01	190	..	5.5	1.0232	0.027
51	316	198	0.204	0.210	2.42	124	56	2.7	1.0373	.....
52	190	257	0.201	0.262	2.29	138	50	3.5	1.0296	.....
53	244	275	0.247	0.266	1.94	186	86	7.0	1.0210	0.040
54	170	275	0.216	0.286	1.81	168	63	8.0	1.0180	0.056
55	184	280	0.184	0.196	2.20	164	48	5.7	1.0241	0.057
56	246	280	0.202	0.212	2.12	152	59	8.0	1.0229	0.056
57	150	280	0.218	0.252	1.98	140	44	6.9	1.0217	0.074
58	168	280	0.194	0.216	2.08	146	65	6.8	1.0218	0.056
59	186	280	0.197	0.202	2.27	150	50	7.5	1.0251	0.092
60	286	280	0.185	0.198	2.24	144	48	5.5	1.0287	0.049
61	220	289	0.229	0.269	2.13	176	76	8.3	1.0207	0.112
62	180	289	0.236	0.213	2.18	124	73	6.4	1.0192	0.040
63	192	289	0.173	0.202	2.38	134	42	3.2	1.0328	.....
64	210	289	0.209	0.256	2.17	150	49	8.4	1.0190	0.070

* Acutely ill.
† Slightly ill.

Normal values for nonprotein nitrogen were obtained in 72 per cent of the rats. The highest value (108 mg.) was seen in an animal acutely ill.

Forty-seven per cent of the animals had values for the $\frac{H.W.}{S.A.}$ ratio above 0.2. The majority had hypertension. The values of $\frac{L.W.}{S.A.}$ ratio varied between 1.44 and 2.49 (average, 2.06). The values of the $\frac{K.W.}{S.A.}$ ratio varied between 0.123 and 0.28 (average, 0.216). Both these average values were fairly representative.

Within sixty days after partial nephrectomy, a number of rats showed well marked hypertension. This is rather striking in view of the long latent period before the appearance of hypertension in the animals receiving the diet that was 10 per cent whole liver.

The second largest percentage of animals with normal urinary output was seen in this group. The output of urinary protein was higher than with the previous diet. The retention of nitrogenous materials was not marked. In other words, on this diet hypertension developed sooner, and evidence of mildly decreased function of the kidney made its appearance.

Twenty Per Cent Whole Liver (Table 5): The urinary volume in 28 per cent of the rats was within normal limits (from 0 to 5 cc.). Slightly increased amounts (from 5 to 8 cc.) were excreted by 39 per cent of this group, and 11 per cent excreted from 8 to 10 cc. A significant effect of this diet was manifested by the increase in the number (18 per cent) excreting between 10 and 15 cc. of urine. Two excreted 17.8 and 18.1 cc. The rats were grouped with respect to specific gravity of the urine as follows: from 1.0100 to 1.0200, 16 per cent; from 1.0200 to 1.0300, 39 per cent; from 1.0300 to 1.0400, 31 per cent, and above 1.0500, 6 per cent. With respect to urinary protein, they were grouped as follows: from 0.000 to 0.050 Gm., 21 per cent; from 0.050 to 0.100 Gm., 35 per cent; from 0.100 to 0.150 Gm., 30 per cent, and from 0.150 to 0.226 Gm., 12 per cent. One animal excreted 0.319 Gm.

Fifty-seven per cent of this group (sixty-one) had normal blood pressure. The first increased blood pressure (170 mm.) was noted on the forty-fourth day. Those with increased blood pressure were grouped as follows: from 140 to 150 mm., 13 per cent; from 150 to 160 mm., 8 per cent; from 160 to 180 mm., 13 per cent, and from 180 to 200 mm., 8 per cent.

The nonprotein nitrogen values continued to remain relatively low. The majority (68 per cent) showed no retention, 25 per cent had slightly increased values (from 60 to 80 mg.), and in only one case was there a value above 100 mg. per hundred cubic centimeters.

The values of the $\frac{H.W.}{S.A.}$ ratio were above 0.2 in 46 per cent of the rats. This ratio did not appear to be related to the blood pressure during the first five months after the operation. Excessively high values (above 0.3) were obtained in three cases. The values of the $\frac{L.W.}{S.A.}$ ratio varied from 1.82 to 2.92. (average, 2.27). Those of the $\frac{K.W.}{S.A.}$ ratio varied from 0.149 to 0.423 (average, 0.252). After the seventy-third day the values were consistently increased.

The addition of a relatively small amount (5 Gm.) of whole dried liver to the preceding diet seemed responsible for an appreciable increase in the degree of polyuria and the excretion of urinary protein. How-

ever, there was little difference in the occurrence and extent of hypertension. The first case of hypertension was seen on the forty-fourth day after operation. After this, high blood pressure was noted at frequent intervals. The animals in this group showed no striking changes in the degree of retention of nitrogen in the blood. The group was characterized by frequent evidence of increased functional impairment and little increase in hypertension.

TABLE 5.—*Observations on Partially Nephrectomized Animals Receiving 20 per Cent Whole Liver*

Rat	Weight, Gm.	Duration of Experiment, Days	Heart Weight / Surface Area ×100	Kidney Weight / Surface Area ×100	Liver Weight / Surface Area ×100	Blood Pressure, Mm.	Nonprotein Nitrogen, Mg. per 100 Cc.	Twenty-Four Hour Urine Concentration Test: Volume Cc.	Specific Gravity	Albumin, Gm.
1	170	14	0.177	0.163	2.32	112	52	4.9	1.0304	.....
2	154	14	0.169	0.173	1.82	140	66	14.0	1.0158	0.024
3	203	14	0.162	0.184	2.38	118	27	4.3	1.0323	.....
4	212	14	0.166	0.172	2.24	124	45	5.3	1.0297	0.017
5	177	14	0.163	0.149	2.06	142	27	3.4	1.0307	.....
6	230	44	0.213	0.261	2.13	120	49	8.7	1.0298	0.111
7	232	44	0.199	0.210	2.09	170	58	7.8	1.0253	0.008
8	121	56	0.162	0.176	....	120	..	1.6	1.0418	.....
9	152	56	0.180	0.208	....	128	..	4.8	1.0339	.....
10	221	60	0.218	0.298	2.68	132	63	14.6	1.0218	0.319
11	269	64	0.185	0.228	2.28	132	50	5.6	1.0299	0.001
12	297	64	0.188	0.203	2.35	136	44	5.4	1.0363	0.020
13	147	69	0.191	0.199	1.95	158	62	7.9	1.0203	0.042
14	121	69	0.312	0.189	2.30	188	87	9.9	1.0197	0.096
15	139	69	0.205	0.210	2.06	140	40	3.6	1.0279	.....
16	202	73	0.210	0.380	2.01	140	62	11.7	1.0169	0.056
17	189	73	0.242	0.242	2.53	200	42	8.6	1.0271	0.147
18	204	73	0.208	0.235	2.75	144	30	5.8	1.0348	0.128
19	290	110	0.236	0.352	2.01	154	85	12.2	1.0241	0.226
20	168	126	0.215	0.272	2.04	194	60	4.5	1.0384	.....
21	221	126	0.326	0.310	1.96	190	93	8.2	1.0250	0.130
22	225	127	0.210	0.228	1.94	200	72	11.0	1.0232	0.122
23	310	144	0.312	0.234	....	170	..	14.7	1.0149	0.074
24	335	157	0.192	0.209	2.46	136	33	4.0	1.0359	.....
25	183	157	0.193	0.279	2.34	154	49	7.7	1.0276	0.173
26	211	157	0.188	0.270	2.42	144	61	7.0	1.0266	0.108
27	232	157	0.185	0.212	2.27	134	31	2.3	1.0343	.....
28	219	158	0.173	0.206	2.23	134	32	2.0	1.0418	.....
29	198	158	0.176	0.214	2.08	126	31	0.6	1.0567	.....
30	349	158	0.173	0.218	2.56	120	32	8.5	1.0444	0.012
31	341	175	0.221	.....	....	174	48	5.5	1.0348	0.114
32	320	180	0.199	0.343	2.24	122	59	6.8	1.0258	0.104
33	311	180	0.175	0.220	1.90	118	30	3.5	1.0334	.....
34	313	180	0.192	0.231	2.01	140	31	2.7	1.0507	.....
35	280	180	0.142	0.202	1.83	126	33	2.5	1.0374	.....
36	247	182	0.211	.....	....	188	57	7.4	1.0270	0.177
37	340	196	0.215	0.389	2.60	164	56	6.5	1.0418	0.117
38	325	196	0.207	0.269	2.36	132	41	8.0	1.0298	0.080
39	214	196	0.226	0.329	2.60	176	90	17.8	1.0153	0.110
40	350	196	0.200	0.272	2.92	114	44	11.2	1.0284	0.202
41	388	197	0.248	0.312	2.15	176	70	14.9	1.0197	0.097
42	210	197	0.157	0.190	1.91	144	34	2.2	1.0419	.....
43	308	197	0.199	0.298	2.56	142	49	5.4	1.0361	0.055
44	318	201	0.202	0.272	2.59	122	59	6.4	1.0339	0.145
45	210	201	0.159	0.200	2.31	136	55	3.7	1.0319	.....
46	234	201	0.183	0.222	2.40	122	52	6.3	1.0289	0.115
47	308	216	0.201	0.248	2.42	144	46	5.5	1.0351	0.101
48	232	216	0.183	0.244	2.19	130	47	9.3	1.0164	0.049
49	365	219	0.211	0.276	2.30	116	64	5.5	1.0334	.....
50	250	220	0.262	0.290	2.35	170	121	18.1	1.0169	0.221
51	318	226	0.211	0.264	2.28	144	69	11.3	1.0210	0.056
52	202	226	0.210	0.423	2.38	156	72	9.9	1.0175	0.062
53	258	226	0.197	0.240	2.03	126	61	12.0	1.0210	0.056
54	246	226	0.256	0.321	2.46	166	76	6.3	1.0222	0.053
55	308	254	0.175	0.319	2.32	130	50	6.3	1.0300	0.072
56	300	254	0.208	0.294	2.31	124	37	2.2	1.0490	.....
57	260	254	0.212	0.330	2.08	138	..	12.0	1.0189	0.060
58	192	254	0.171	0.273	2.28	128	63	6.0	1.0231	0.090
59	276	254	0.202	0.239	2.33	152	59	7.1	1.0237	0.063
60	315	254	0.198	0.253	2.53	120	..	7.3	1.0274	0.033
61	274	254	0.201	0.248	2.30	146	55	5.9	1.0332	0.080

Forty Per Cent Whole Liver (Table 6): The urinary volume was within the normal range in 12 per cent of the animals. The rats secreting amounts greater than normal were grouped as follows: from 5 to 10 cc., 31 per cent; from 10 to 15 cc., 39 per cent, and from 15 to 20 cc., 15 per cent. Two rats excreted 20.7 and 21 cc. According to the specific gravity of the urine, the groups were: from

TABLE 6.—*Observations on Partially Nephrectomized Animals Receiving 40 per Cent Whole Liver*

Rat	Weight, Gm.	Duration of Experiment, Days	Heart Weight / Surface Area ×100	Kidney Weight / Surface Area ×100	Liver Weight / Surface Area ×100	Blood Pressure, Mm.	Nonprotein Nitrogen, Mg. per 100 Cc.	Twenty-Four Hour Urine Concentration Test: Volume Cc.	Specific Gravity	Albumin, Gm.
1	222	14	0.175	0.188	2.07	162	69	14.3	1.0299	0.020
2	210	17	0.152	0.205	2.18	118	68	11.2	1.0239	0.022
3	179	17	0.161	0.202	2.40	116	59	4.5	1.0368	.....
4	180	17	0.153	0.216	2.05	126	57	3.5	1.0364	.....
5	149	17	0.138	0.151	2.33	126	60	5.4	1.0315	0.015
6	195	17	0.151	0.173	2.29	114	53	9.1	1.0298	0.017
7	105	17	0.129	0.116	2.16	120	63	7.9	1.0236	0.008
8	299	44	0.213	0.385	3.54	126	56	8.5	1.0317	0.080
9	203	44	0.208	0.295	2.91	124	97	19.0	1.0196	0.213
10	206	44	0.223	0.313	3.24	132	56	4.5	1.0334	.....
11	250	44	0.187	0.323	2.94	138	77	11.3	1.0252	0.119
12	215	47	0.184	0.304	2.29	164	71	8.3	1.0252	0.140
13	256	47	0.174	0.258	2.41	126	44	5.7	1.0413	0.018
14	214	47	0.159	0.286	2.24	140	45	5.4	1.0379	.....
15	157	47	0.203	0.271	2.04	174	80	9.5	1.0231	0.104
16	209	47	0.187	0.313	2.22	126	82	11.4	1.0267	0.187
17	261	50	0.208	0.285	2.78	128	63	5.8	1.0367	.....
18	234	50	0.190	0.266	2.77	130	58	3.2	1.0434	.....
19	240	50	0.180	0.260	2.50	132	53	4.5	1.0390	.....
20	247	51	0.227	0.468	2.34	152	89	13.9	1.0258	0.202
21	227	72	0.240	0.280	2.35	186	125	20.7	1.0136	0.093
22	175	72	0.225	0.420	2.53	176	91	17.0	1.0195	0.372
23	241	72	0.204	0.365	2.31	158	98	12.7	1.0190	0.085
24	199	78	0.191	0.360	2.46	150	64	11.4	1.0180	0.065
25	193	78	0.179	0.272	2.28	114	86	13.2	1.0192	0.136
26	293	87	0.281	0.744	2.47	212	106	17.6	1.0200	0.186
27	210	87	0.198	0.464	2.92	146	70	11.3	1.0207	0.127
28	197	92	0.181	0.319	1.92	134	100	11.9	1.0187	0.033
29	183	92	0.222	0.392	2.82	168	104	10.7	1.0212	0.120
30	212	92	0.264	0.618	2.78	178	104	13.5	1.0202	0.114
31	237	92	0.234	0.372	2.55	170	88	10.0	1.0272	0.145
32	153	92	0.255	0.623	3.47	144	119	13.7	1.0193	0.163
33	208	112	0.185	0.256	2.91	134	58	11.7	1.0179	0.059
34	311	125	0.309	0.382	2.68	150	63	12.0	1.0235	0.093
35	189	142	0.248	0.408	2.39	130	90	11.5	1.0227	0.002
36	373	148	0.204	0.435	2.85	154	71	16.4	1.0229	0.080
37	200	148	0.222	0.568	3.28	176	132	13.5	1.0192	0.184
38	295	148	0.203	0.442	2.47	132	89	17.7	1.0200	0.186
39	289	160	0.182	0.251	2.49	138	44	5.8	1.0308	0.066
40	321	160	0.171	0.304	2.77	136	59	8.8	1.0298	0.188
41	271	160	0.191	0.364	2.59	136	62	3.8	1.0265	0.183
42	227	160	0.185	0.446	2.33	180	103	11.6	1.0229	0.204
43	345	160	0.234	1.020	2.18	196	145	18.2	1.0155	0.078
44	304	160	0.230	0.518	2.39	156	106	15.8	1.0117	0.163
45	255	160	0.178	0.290	1.98	154	105	21.0	1.0136	0.059
46	330	184	0.193	0.467	2.38	136	86	10.0	1.0251	0.214

1.0100 to 1.0200, 28 per cent; from 1.0200 to 1.0300, 48 per cent, and from 1.0300 to 1.0400, 20 per cent. Two animals excreted urine with specific gravity above 1.400. According to urinary protein, the groups were as follows: from 0.000 to 0.05 Gm., 21 per cent; from 0.05 to 0.1 Gm., 26 per cent; from 0.1 to 0.15 Gm., 21 per cent; from 0.15 to 0.2 Gm., 20 per cent, and from 0.2 to 0.214 Gm., 10 per cent, and one rat excreted 0.372 Gm.

Animals with normal blood pressure comprised 54 per cent of this group (forty-six). The first rat with hypertension was seen on the fourteenth day, and the next on the forty-seventh day. The animals with hypertension were grouped

as follows: from 140 to 160 mm., 20 per cent; from 160 to 180, 20 per cent, and three had pressures above 180 mm.

Fewer animals (28 per cent) in this group had a normal concentration of nonprotein nitrogen than in the preceding groups. Those with retention were grouped as follows: from 60 to 80 mg., 28 per cent; from 80 to 100 mg., 22 per cent, and from 100 to 150 mg., 22 per cent.

The $\frac{H.W.}{S.A.}$ ratio was normal in 46 per cent of this group. There was a poor relationship between the $\frac{H.W.}{S.A.}$ ratio and the blood pressure. The values of the $\frac{L.W.}{S.A.}$ ratio varied between 1.92 and 3.42 (average, 2.52). The values of the $\frac{K.W.}{S.A.}$ ratio varied between 0.116 and 1.02 (average, 0.361).

A striking contrast between the rats receiving this diet and those receiving the preceding diets was manifested in the change in urinary volume. There was a definite and rather marked increase in the volume of urine excreted during the concentration test. The specific gravity of these specimens was lower. The albumin excreted was only slightly greater. Retention of nitrogen was more marked and frequent in those receiving the diet richer in nitrogen. Despite the fact that the renal function appeared to be more seriously damaged in these rats, there was little effect on the occurrence, incidence and distribution of hypertension.

Sixty Per Cent Whole Liver (Table 7): The volume of urine excreted was within normal range in 24 per cent of the animals. Those with volumes above normal were grouped as follows: from 5 to 10 cc., 20 per cent; from 10 to 15 cc., 34 per cent, and from 15 to 20 cc., 20 per cent. One animal excreted 20.5 cc. With respect to the specific gravity of the urine the rats were grouped as follows: from 1.0100 to 1.0200, 32 per cent; from 1.0200 to 1.0300, 38 per cent; from 1.0300 to 1.0400, 28 per cent. The urine of one animal had a specific gravity above 1.0400. With respect to the urinary protein excreted, the rats were grouped as follows: from 0.000 to 0.05 Gm., 23 per cent; from 0.05 to 0.1 Gm., 28 per cent; from 0.1 to 0.15 Gm., 21 per cent; from 0.150 to 0.2 Gm., 21 per cent, and from 0.2 to 0.25 Gm., 7 per cent.

Animals with normal blood pressure comprised 45 per cent of this group (fifty-one). The first rat with hypertension was noted on the twenty-third day after the operation, and increased blood pressure was seen at frequent intervals after this. The rats with hypertension were grouped as follows: from 140 to 160 mm., 31 per cent; from 160 to 180 mm., 16 per cent, and from 180 to 200 mm., 8 per cent.

Twenty-eight per cent of this group had concentrations of nonprotein nitrogen within the normal range. Those with retention were grouped as follows: from 60 to 80 mg., 16 per cent; from 80 to 100 mg., 18 per cent, and from 100 to 150 mg., 34 per cent. Two rats had concentrations of 186 and 174 mg., respectively.

The values of the $\frac{H.W.}{S.A.}$ ratio were above 0.2 in 33 per cent of the cases. There was a poor relationship between the $\frac{H.W.}{S.A.}$ ratio and blood pressure up to the ninety-second day. The values of the $\frac{L.W.}{S.A.}$ ratio varied between 1.85 and 3.28 (average, 2.52). The values of the $\frac{K.W.}{S.A.}$ ratio varied between 0.177 and 0.71 (average, 0.41).

The only significant difference present in this group was in the increased frequency of retention of nitrogen. This fact could not be correlated with the damage to the kidney as judged by the concentra-

TABLE 7.—*Observations on Partially Nephrectomized Animals Receiving 60 per Cent Whole Liver*

Rat	Weight, Gm.	Duration of Experiment, Days	Heart Weight / Surface Area ×100	Kidney Weight / Surface Area ×100	Liver Weight / Surface Area ×100	Blood Pressure, Mm.	Nonprotein Nitrogen, Mg. per 100 Cc.	Twenty-Four Hour Urine Concentration Test: Volume Cc.	Specific Gravity	Albumin, Gm.
1	156	15	0.171	0.209	2.72	112	96	7.7	1.0283	0.035*
2	200	15	0.173	0.261	2.20	104	129	20.5	1.0153	0.018*
3	155	23	0.149	0.197	1.85	130	123	10.2	1.0204	0.025*
4	141	23	0.160	0.215	2.39	138	49	4.2	1.0363*	.....
5	161	23	0.162	0.238	2.25	156	119	4.2	1.0411*	.....
6	123	23	0.141	0.177	2.02	134	85	8.0	1.0241	0.111*
7	149	23	0.160	0.229	2.20	122	110	8.1	1.0271	0.068*
8	201	39	0.194	0.345	2.58	170	98	14.5	1.0229	0.194
9	188	39	0.169	0.290	2.60	116	66	7.0	1.0337	0.086
10	195	39	0.171	0.220	2.70	126	44	10.1	1.0248	0.008
11	170	41	0.180	0.404	2.54	144	98	13.1	1.0207	0.157*
12	198	47	0.194	0.300	2.76	156	51	7.4	1.0344	0.075*
13	201	47	0.193	0.373	2.95	136	50	11.6	1.0274	0.192*
14	202	69	0.162	0.416	2.11	148	104	13.9	1.0169	0.049
15	267	69	0.187	0.562	2.21	142	95	11.9	1.0217	0.069
16	178	92	0.253	0.710	2.25	190	132	14.0	1.0165	.....
17	189	93	0.213	0.571	2.78	148	88	10.0	1.0241	0.235
18	174	102	0.213	0.480	2.40	146	126	11.2	1.0154	0.049
19	258	105	0.219	0.790	2.59	166	186	19.2	1.0154	0.136
20	285	107	0.199	0.218	2.54	146	71	16.5	1.0245	0.197
21	196	112	0.196	.....	....	140	..	6.8	1.0262	0.060
22	311	117	0.207	0.660	2.61	138	85	...	.....	.....
23	253	128	0.234	0.546	2.42	154	104	14.5	1.0174	0.080
24	191	128	0.224	0.373	2.20	186	109	14.0	1.0172	0.094
25	303	128	0.246	0.425	2.54	152	127	15.8	1.0169	.....
26	270	133	0.187	0.409	2.90	120	66	8.6	1.0300	0.118
27	183	134	0.219	0.341	2.79	118	72	5.5	1.0322	0.025
28	203	134	0.185	0.484	2.76	172	83	8.5	1.0263	0.125
29	170	134	0.194	0.339	2.62	138	56	4.5	1.0295	.....
30	201	134	0.208	0.527	2.54	190	119	15.0	1.0193	0.149
31	225	134	0.169	0.311	2.63	120	47	3.3	1.0398	.....
32	162	139	0.165	0.302	2.11	150	143	10.5	1.0177	0.028
33	207	142	0.193	0.370	2.67	156	68	5.5	1.0342	0.091
34	203	142	0.194	0.598	2.58	172	148	19.6	1.0162	0.168
35	200	142	0.202	0.391	2.38	130	71	5.1	1.0300	0.074
36	300	150	0.311	0.637	2.79	142	65	13.5	1.0243	0.177
37	308	150	0.217	0.830	2.81	170	106	16.0	1.0211	0.205
38	304	150	0.192	0.528	2.74	138	50	10.8	1.0271	0.138
39	218	150	0.222	0.321	2.67	136	42	3.9	1.0398	.....
40	242	185	0.199	0.562	2.13	162	104	15.1	1.0188	0.153
41	263	185	0.184	0.587	2.12	190	118	18.7	1.0197	0.196
42	226	185	0.174	0.423	2.44	164	69	10.6	1.0247	0.146
43	257	185	0.168	0.302	2.52	154	48	6.5	1.0313	0.026
44	152	185	0.193	0.366	2.27	176	174	11.8	1.0165	0.071
45	238	185	0.276	0.470	2.29	154	118	18.6	1.0196	0.227
46	190	188	0.151	0.252	2.04	124	58	7.7	1.0225	0.073
47	296	188	0.252	0.540	2.68	132	83	16.6	1.0196	0.163
48	290	188	0.191	0.398	3.01	120	55	5.6	1.0397	0.122
49	358	188	0.173	0.341	3.28	120	44	5.6	1.0361	0.018
50	228	188	0.201	0.362	2.84	124	50	4.6	1.0383	0.111
51	219	188	0.181	0.310	2.95	120	47	5.3	1.0383	0.054

* One day concentration test.

tion test. There was a slight increase in the percentage of animals having increased blood pressure.

Eighty Per Cent Whole Liver Diet (Table 8): The volume of urine was within normal limits in 2 per cent of the rats. Rats with volume above normal were grouped as follows: from 5 to 10 cc., 35 per cent; from 10 to 15 cc., 39 per cent; from 15 to 20 cc., 21 per cent, and two rats excreted 20.7 and 22.1 cc., respectively.

With respect to the specific gravity of the urine, the rats were grouped as follows: from 1.0100 to 1.0200, 43 per cent; from 1.0200 to 1.0300, 48 per cent, and from 1.0300 to 1.0400, 9 per cent. With respect to the urinary protein excreted, they were grouped as follows: from 0.000 to 0.50 Gm., 60 per cent; from 0.50 to 0.100 Gm., 23 per cent; from 0.100 to 0.150 Gm., 13 per cent, and from 0.150 to 0.200 Gm., 4 per cent.

TABLE 8.—*Observations on Partially Nephrectomized Animals Receiving 80 per Cent Whole Liver*

Rat	Weight, Gm.	Duration of Experiment, Days	Heart Weight / Surface Area ×100	Kidney Weight / Surface Area ×100	Liver Weight / Surface Area ×100	Blood Pressure, Mm.	Nonprotein Nitrogen, Mg. per 100 Cc.	Twenty-Four Hour Urine Concentration Test: Volume Cc.	Specific Gravity	Albumin, Gm.
1	144	14	0.130	0.166	2.05	120	126	...	.....	.....
2	176	14	0.136	0.202	1.97	112	...	...	.....	.....
3	128	20	0.136	0.295	2.36	124	78	8.7	1.0305	0.007*
4	120	27	0.151	0.216	2.44	120	166	8.7	1.0259	0.035*
5	106	27	0.153	0.266	2.61	124	...	7.5	1.0272	0.013*
6	138	27	0.146	0.264	2.40	122	160	8.5	1.0250	0.034*
7	190	27	0.143	0.343	2.27	120	116	15.0	1.0232	0.038*
8	106	27	0.130	0.326	2.21	126	110	7.6	1.0230	0.006*
9	120	27	0.141	0.284	2.38	124	105	11.5	1.0175	0.006*
10	164	31	0.150	0.354	2.34	112	180	9.0	1.0226	0.018*
11	188	31	0.145	0.277	2.08	130	138	9.5	1.0273	0.010*
12	164	31	0.158	0.227	2.18	126	197	15.6	1.0213	0.027*
13	152	31	0.157	0.210	1.96	116	176	12.0	1.0193	0.019*
14	168	31	0.150	0.339	2.12	118	196	16.7	1.0228	.....
15	168	32	0.141	0.278	2.03	126	117	....	.....	.....
16	188	39	0.154	0.333	2.23	138	119	17.6	1.0239	0.046*
17	162	39	....	0.313	2.30	116	...	11.1	1.0254	0.035*
18	178	39	0.148	0.307	2.04	128	116	17.7	1.0222	0.030*
19	98	40	0.170	0.368	2.50	160	160	10.1	1.0222	0.039*
20	167	40	0.163	0.448	2.53	156	115	14.2	1.0241	0.146*
21	116	40	0.170	0.332	2.43	152	185	12.2	1.0234	0.059*
22	139	40	0.159	0.344	2.74	144	83	5.5	1.0333	0.011*
23	176	47	0.185	0.391	2.91	146	100	16.3	1.0218	.....
24	195	47	0.167	0.489	3.25	140	78	8.4	1.0282	.....
25	274	68	0.197	0.898	3.74	146	80	17.8	1.0224	0.123*
26	103	69	0.208	0.315	2.14	158	119	4.5	1.0195	0.009
27	187	69	0.198	0.523	2.68	148	123	14.2	1.0168	0.040
28	211	69	0.205	0.710	2.74	150	202	20.7	1.0150	0.083
29	227	72	0.248	0.905	2.61	194	156	13.0	1.0175	0.072
30	180	87	0.182	0.698	3.13	138	123	16.4	1.0169	0.103
31	122	91	0.175	0.374	....	168	...	7.6	1.0226	0.063
32	129	102	0.232	0.528	....	180	170	12.4	1.0176	0.081
33	300	113	0.229	0.527	2.78	158	74	16.4	1.0192	0.043
34	260	113	0.204	0.519	2.47	158	81	16.9	1.0174	0.038
35	185	116	0.203	0.514	2.62	156	132	12.8	1.0241	.....
36	255	117	0.202	0.639	2.41	146	149	13.8	1.0153	0.015
37	337	117	0.198	0.483	2.79	138	67	9.5	1.0262	0.030
38	130	120	0.166	0.253	2.53	140	161	10.7	1.0206	0.047
39	87	120	0.202	0.537	2.76	140	237	10.0	1.0191	0.102
40	172	120	0.194	0.582	2.78	132	222	14.9	1.0171	0.094
41	169	120	0.186	0.428	2.68	144	132	14.2	1.0189	0.044
42	345	126	0.229	0.640	3.09	130	127	18.6	1.0204	0.117
43	292	126	0.228	0.597	2.55	148	100	15.8	1.0202	0.103
44	121	150	0.192	0.375	1.98	122	193	8.7	1.0177	0.017
45	137	150	0.186	0.356	2.37	138	132	...	.....	.....
46	138	150	0.160	0.402	2.37	120	134	9.9	1.0181	0.048
47	146	150	0.219	0.722	2.35	190	242	14.3	1.0157	0.038
48	200	150	0.182	0.433	2.28	124	107	13.4	1.0182	0.075
49	172	151	0.189	0.457	2.32	178	78	9.3	1.0195	.....
50	181	151	0.168	0.391	2.65	122	65	5.4	1.0353	.....
51	146	151	0.140	0.527	2.20	154	135	12.5	1.0174	0.064
52	230	153	0.183	0.440	2.58	120	92	8.6	1.0307	0.039
53	163	153	0.160	0.438	2.77	120	..	10.0	1.0261	0.080
54	130	153	0.188	0.321	2.69	124	98	14.1	1.0164	0.057
55	214	153	0.183	0.396	2.53	120	161	22.1	1.0162	.....
56	230	174	0.177	0.482	3.47	130	60	5.8	1.0386	0.057
57	310	174	0.204	1.010	3.10	128	198	13.3	1.0224	0.182
58	308	174	0.198	1.170	3.21	124	150	12.5	1.0271	0.232
59	248	200	0.206	0.795	3.26	168	157	13.0	1.0189	0.156
60	132	204	0.222	0.422	2.51	190	...	18.6	1.0134	0.022

* One day concentration test.

There was a large number of animals (62 per cent) with normal blood pressure. This may be due to the larger percentage of younger animals studied in this group. The first hypertensive rat was seen on the fortieth day after operation. The hypertensive animals were grouped as follows: from 140 to 150 mm., 13 per cent; from 150 to 160 mm., 13 per cent, and from 160 to 194 mm., 12 per cent.

Seven per cent of the animals in this group had normal nonprotein nitrogen concentration. Those with retention were grouped as follows: from 75 to 100 mg., 19 per cent; from 100 to 150 mg., 39 per cent; from 150 to 200 mg., 28 per cent, and from 200 to 250 mg., 7 per cent.

The values of the $\frac{H.W.}{S.A.}$ ratio were increased in 24 per cent of the animals. The values of the $\frac{L.W.}{S.A.}$ ratio varied between 1.93 and 3.26 (average, 2.54). The values of the $\frac{K.W.}{S.A.}$ ratio varied between 0.166 and 1.17 (average, 0.453).

This diet caused a still further increase in the retention of nitrogen. The percentage of animals having polyuria during the concentration test was slightly greater than that of any of those receiving the preceding diets. The number of rats with hypertension within a given period after operation seemed to be smaller than in the groups receiving the diets containing smaller quantities of liver. There was a diminution in the amount of urinary protein excreted by these animals. The values of the $\frac{H.W.}{S.A}$ ratio were smaller for the animals of this group than for those of the same age in the previously described dietary groups, particularly as concerned younger animals. Despite the comparatively high mortality and increased renal damage, the toxicity of the diet was not reflected in any striking effect on the blood pressure.

Eighty Per Cent Liver Residue (Table 9): Twenty-six per cent of this group had normal urinary volume. Those with increased volume were grouped as follows: from 5 to 10 cc., 34 per cent; from 10 to 15 cc., 26 per cent; from 15 to 20 cc., 10 per cent, and 2 rats excreted 22.7 and 23.1 cc., respectively. With respect to the specific gravity, the rats were grouped as follows: from 1.0100 to 1.0200, 15 per cent; from 1.0200 to 1.0300, 58 per cent; from 1.0300 to 1.0400, 21 per cent, and from 1.0400 to 1.0600, 6 per cent. The amount of urinary protein was less than that excreted by those receiving diets L. 20, L. 40 and L. 60, as shown by the following grouping: from 0.000 to 0.05 Gm., 67 per cent; from 0.05 to 0.1 Gm., 10 per cent; from 0.1 to 0.2 Gm., 12 per cent, and from 0.2 to 0.25 Gm., 11 per cent.

An unusually large number had a blood pressure below 120 mm. (27 per cent), and 58 per cent had a blood pressure between 120 and 140 mm., and 85 per cent of the total number had a blood pressure within normal limits. A slight increase was noted in 13 per cent, and 2 rats had blood pressures of 152 and 172 mm., respectively.

Normal nonprotein nitrogen concentration was seen in 23 per cent of the rats. Those with retention were grouped as follows: from 100 to 150 mg., 34 per cent; from 150 to 200 mg., 25 per cent; from 200 to 250 mg., 9 per cent, and from 250 to 300 mg., 9 per cent.

The values of the $\frac{H.W.}{S.A.}$ ratio were elevated in 17 per cent of the group. The values of the $\frac{L.W.}{S.A.}$ ratio varied between 1.87 and 3.18 (average, 2.42). The values of the $\frac{K.W.}{S.A.}$ ratio varied from 0.163 to 1.06 (average, 0.534).

The response to this diet was unexpected and difficult to explain. The large number of animals with retention of nitrogen, polyuria and lowered specific gravity was evidence of an appreciable loss of renal function. However, despite this damage, few animals showed excessive

TABLE 9.—*Observations on Partially Nephrectomized Animals Receiving 80 per Cent Liver Residue*

Rat	Weight, Gm.	Duration of Experiment, Days	Heart Weight / Surface Area ×100	Kidney Weight / Surface Area ×100	Liver Weight / Surface Area ×100	Blood Pressure, Mm.	Nonprotein Nitrogen, Mg. per 100 Cc.	Twenty-Four Hour Urine Concentration Test: Volume Cc.	Specific Gravity	Albumin, Gm.
1	181	30	0.162	0.396	2.60	124	121	13.8	1.0221	0.026
2	124	30	0.150	0.311	2.60	122	112	4.2	1.0324	.....
3	80	30	0.147	0.258	1.94	104	...	2.7	1.0354	.....
4	159	41	0.141	0.300	2.16	116	...	11.8	1.0236	0.034
5	170	41	0.166	0.288	2.23	110	132	9.7	1.0275	0.018
6	148	41	0.151	0.281	2.30	118	...	4.0	1.0515	.....
7	146	41	0.165	0.343	1.82	124	200	13.2	1.0299	0.013
8	160	66	0.151	0.335	2.09	120	120	8.0	1.0253	0.008
9	122	66	0.140	0.386	2.01	130	125	5.0	1.0303	.....
10	124	66	0.144	0.366	1.87	148	161	4.0	1.0286	.....
11	136	73	0.153	0.317	2.24	128	208	5.8	1.0254	.....
12	133	73	0.141	0.287	2.21	116	138	2.2	1.0354	.....
13	100	73	0.132	0.163	2.10	126	143	2.1	1.0344	.....
14	176	73	0.147	0.289	2.31	108	114	4.4	1.0342	.....
15	161	95	0.161	0.356	2.41	136	58	7.0	1.0311	0.011
16	132	95	0.149	0.400	1.97	130	67	3.7	1.0310	.....
17	157	95	0.154	0.435	2.38	144	66	8.5	1.0251	0.010
18	158	95	0.146	0.364	2.35	138	46	6.7	1.0298	0.011
19	144	95	0.161	0.503	2.19	124	96	9.0	1.0219	0.010
20	215	96	0.183	0.508	....	130	135	13.0	1.0259	0.091
21	180	105	0.226	0.535	....	136	160	16.0	1.0247	0.001
22	248	112	0.183	0.421	2.78	152	55	9.2	1.0282	.....
23	238	120	0.227	0.990	2.74	142	270	22.7	1.0175	0.168
24	336	120	0.184	0.648	3.18	126	106	9.2	1.0282	0.034
25	218	125	0.255	1.060	2.76	172	245	15.0	1.0175	0.192
26	235	125	0.267	0.945	2.56	130	182	14.5	1.0200	0.086
27	147	128	0.172	0.402	....	110	127	4.1	1.0326	.....
28	174	128	0.191	0.532	2.34	104	163	9.2	1.0234	0.017
29	282	149	0.234	0.915	2.68	148	227	23.1	1.0152	0.219
30	242	149	0.184	0.628	2.44	130	114	14.5	1.0197	0.042
31	186	154	0.199	0.894	....	134	183	11.9	1.0202	0.120
32	194	163	0.210	0.760	2.79	132	163	11.7	1.0212	0.206
33	240	163	0.186	0.672	2.63	132	...	9.1	1.0239	0.068
34	206	163	0.247	1.050	2.40	140	262	19.5	1.0152	0.143
35	160	169	0.195	0.930	2.13	126	288	17.2	1.0127	0.024
36	148	175	0.168	0.492	2.45	122	95	3.5	1.0437	.....
37	236	182	0.176	0.448	2.80	148	64	4.4	1.0466	0.000
38	250	182	0.195	0.965	2.81	134	148	14.9	1.0211	0.212
39	214	183	0.187	0.545	3.48	122	59	4.6	1.0322	0.038
40	152	194	0.194	0.557	2.30	120	152	15.3	1.0187	0.011
41	136	194	0.188	0.670	2.08	132	193	9.7	1.0241	0.038
42	136	195	0.217	0.902	1.88	112	282	...	.....	.....
43	118	216	0.181	0.433	2.30	118	180	7.6	1.0236	0.013
44	134	226	0.157	0.344	2.27	116	158	5.6	1.0272	.....
45	152	226	0.169	0.457	2.84	134	154	10.8	1.0232	0.046
46	208	226	0.180	0.483	2.44	122	106	11.8	1.0236	0.028
47	246	226	0.185	0.543	2.80	128	149	12.3	1.0274	0.040
48	170	226	0.172	0.558	2.69	132	99	9.7	1.0245	0.083

excretion of protein or hypertension. It should be pointed out that although this diet was the richest in nitrogen content, the mortality rate was not so great as that of the animals fed the diets containing whole liver with a much smaller concentration of nitrogen.

Diet Rich in the Water-Soluble Constituents of Liver (Table 10): The urinary volume was normal in 22 per cent of the animals. Those with increased volume were grouped as follows: from 5 to 10 cc., 39 per cent; from 10 to 15 cc., 37 per cent, and two rats excreted 19.2 and 20.6 cc., respectively. With respect

to the specific gravity, the rats were grouped as follows: from 1.0100 to 1.0200, 17 per cent; from 1.0200 to 1.0300, 59 per cent; from 1.0300 to 1.0400, 22 per cent, and one rat had a specific gravity of 1.0410. With respect to the amount of urinary protein excreted, they were grouped as follows: from 0.000 to 0.50 Gm., 27 per cent; from 0.05 to 0.1 Gm., 30 per cent; from 0.1 to 0.15 Gm., 20 per cent; from 0.150 to 0.2 Gm., 17 per cent, and two rats excreted 0.214 and 0.216 Gm., respectively.

TABLE 10.—*Observations on Partially Nephrectomized Animals Receiving a Diet Rich in Aqueous Liver Extract*

Rat	Weight, Gm.	Duration of Experiment, Days	Heart Weight / Surface Area ×100	Kidney Weight / Surface Area ×100	Liver Weight / Surface Area ×100	Blood Pressure, Mm.	Nonprotein Nitrogen, Mg. per 100 Cc.	Twenty-Four Hour Urine Concentration Test: Volume Cc.	Specific Gravity	Albumin, Gm.
1	134	15	0.145	0.229	1.99	126	85	3.8	1.0300	.....
2	180	15	0.157	0.260	2.45	128	87	6.4	1.0265	.....
3	165	15	0.155	0.302	2.33	124	111	8.7	1.0228	.....
4	165	15	0.158	0.255	2.46	126	..	4.4	1.0232	.....
5	222	28	0.218	0.331	2.70	152	77	11.0	1.0255	0.082
6	157	28	0.210	0.288	2.33	116	99	6.7	1.0295	.....
7	166	28	0.176	0.380	2.71	114	92	5.0	1.0289	0.029
8	172	28	0.186	0.301	2.58	120	65	...	.....	.....
9	161	39	0.161	0.271	2.48	126	66	5.8	1.0294	0.028
10	170	39	0.163	0.256	2.48	128	55	5.2	1.0305	0.004
11	184	39	0.178	0.244	2.32	120	64	7.0	1.0273	0.037
12	169	39	0.178	0.258	2.44	124	46	5.6	1.0281	0.016
13	240	85	0.208	0.396	2.44	122	95	11.2	1.0300	0.142
14	181	85	0.198	0.423	2.91	124	86	10.4	1.0257	0.094
15	164	112	0.186	0.296	2.63	128	92	10.3	1.0209	0.149
16	140	112	0.222	0.348	2.34	170	111	14.0	1.0193	0.122
17	162	112	0.183	0.381	2.46	170	101	11.5	1.0181	0.135
18	298	124	0.234	0.410	2.22	168	80	11.6	1.0260	0.099
19	280	124	0.238	0.391	2.44	176	76	19.2	1.0188	0.158
20	176	124	0.258	0.396	2.28	184	116	14.8	1.0176	0.092
21	287	125	0.219	0.380	2.28	152	45	10.4	1.0323	0.144
22	367	125	0.226	0.360	2.91	140	44	10.7	1.0312	0.109
23	300	131	0.213	0.321	1.90	130	57	8.6	1.0238	0.053
24	276	131	0.180	0.268	1.87	144	..	4.1	1.0273	.....
25	280	131	0.176	0.334	2.10	142	47	4.6	1.0334	.....
26	215	142	0.218	0.315	2.12	138	83	12.8	1.0189	0.092
27	179	142	0.196	0.308	2.58	...	56	6.9	1.0292	0.036
28	158	142	0.230	0.380	2.71	160	98	11.4	1.0191	0.198
29	274	147	0.175	0.334	2.07	118	37	3.1	1.0410	.....
30	322	147	0.174	0.369	2.27	135	53	5.9	1.0314	0.040
31	236	147	0.169	0.392	2.36	146	47	4.2	1.0352	.....
32	214	147	0.170	0.324	1.72	114	51	4.4	1.0267	0.002
33	226	147	0.155	0.264	1.90	128	46	4.1	1.0283	.....
34	300	157	0.223	0.481	2.44	134	67	8.0	1.0252	.....
35	211	164	0.201	0.390	3.70	124	104	8.9	1.0212	0.068
36	185	164	0.229	0.383	2.71	190	129	10.0	1.0238	0.172
37	188	164	0.226	0.320	2.85	158	112	12.9	1.0190	0.194
38	154	176	0.163	0.275	2.08	120	80	7.9	1.0227	0.081
39	232	176	0.167	0.392	2.35	162	59	5.1	1.0305	0.075
40	224	176	0.196	0.392	2.53	144	84	20.6	1.0225	0.214
41	265	176	0.217	0.333	2.25	164	73	12.0	1.0225	0.168
42	200	191	0.183	0.407	3.60	154	55	9.4	1.0281	0.216

Fifty-six per cent of this group had blood pressures within normal limits. Those with elevated blood pressures were grouped as follows: from 140 to 150 mm., 12 per cent; from 150 to 160 mm., 10 per cent; from 160 to 180 mm., 17 per cent, and from 180 to 200 mm., 5 per cent.

Normal nonprotein nitrogen concentrations were seen in 17 per cent of the animals. Those with retention were grouped as follows: from 50 to 100 mg., 63 per cent, and from 100 to 150 mg., 17 per cent.

The values of the $\frac{\text{H.W.}}{\text{S.A.}}$ ratio were elevated in 41 per cent. The values of the $\frac{\text{L.W.}}{\text{S.A.}}$ ratio varied between 1.72 and 3.70 (average, 2.41). Those of the $\frac{\text{K.W.}}{\text{S.A.}}$ ratio ranged between 0.229 and 0.491 (average, 0.336).

This diet caused the greatest mortality among the experimental animals. Despite the apparent toxicity, the concentration test did not indicate severe renal insufficiency, nor was the albuminuria particularly marked, and the retention of nitrogen was only moderately elevated. The frequency and the degree of hypertension were no more marked than in animals fed moderate or large quantities of whole liver.

ANALYSIS OF OBSERVATIONS

If graded amounts of a nephrotoxic material are added to the diet of partially nephrectomized rats, the effects should be manifested to a certain degree in the volume of urine, the ability to concentrate urine, the amount of protein in the urine, the blood pressure, azotemia and

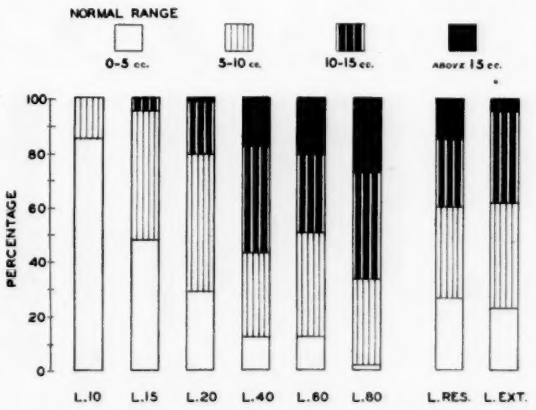

Chart 1.—The relation of the urinary volume to diet in partially nephrectomized rats.

the weights of the heart, liver and kidney. The effects of the various diets containing liver on each of these functions and on the organs studied are discussed.

Urine Concentration Test.—The ability of the organism to retain fluid during a period of abstinence from food and water has been utilized as a test of renal function. Intact and unilaterally nephrectomized animals secrete small amounts of concentrated urine. Chart 1 shows in diagrammatic form the effects of the various diets on the urinary volume of partially nephrectomized animals. The progressive increase in the incidence and the degree of polyuria with graded increments of whole liver is striking. Mild polyuria in 15 per cent of the rats receiving diet L. 10 offers a strong contrast to the 98 per cent which secreted more than the normal amount when receiving diet L. 80. The volume

of urine excreted by the animals receiving the L. Res. and L. Ext. diets was between those obtained from groups receiving L. 20 and L. 40 diets.

Chart 2 shows the effect of the same diets on the specific gravity of the urine. It will be seen that the ability to concentrate in general varies inversely with the percentage of whole liver in the diet. The regular increase in the percentage of animals (represented by solid black) with hyposthenuria is noteworthy. In only a small number of animals receiving the L. Res. and L. Ext. diets was the concentration normal.

It is interesting that few (18 per cent) receiving the L. 10 diet had normal concentration whereas most (85 per cent) receiving the same diet excreted a normal amount. The volume of urine excreted by many

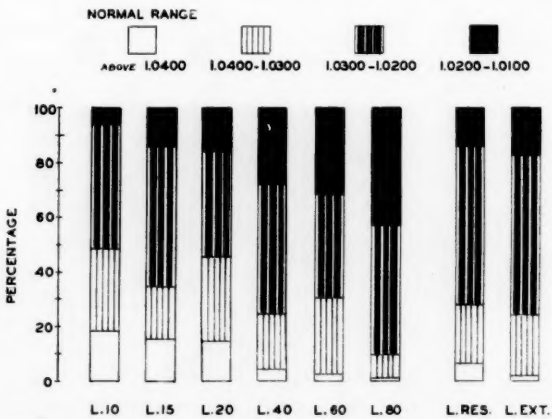

Chart 2.—The relation of specific gravity to diet in partially nephrectomized rats.

of these animals, without food and water, exceeded their blood volume (about 20 cc. in a rat weighing 300 Gm.).

Albuminuria.—Chart 3 summarizes the effects of the diets on the excretion of protein. Freedom from abnormal albuminuria in the animals receiving the L. 10 diet was outstanding, as was the fact that here again the animals fed the L. 80 and L. Res. diets excreted less than the groups fed diets L. 60, L. 40 and L. 20. The most striking albuminuria was seen in the diet group receiving L. 40, with those receiving diets L. 20, L. 60 and L. Ext. having about an equal amount. There were marked variations in the amount of protein excreted by the individual members of each group. This seemed independent of the time elapsed after operation and the volume of urine excreted.

Hypertension.—Chart 4 summarizes the effects of the diets on the blood pressure. The absence of significant hypertension (over 160 mm.) in those receiving the L. Res. diet was remarkable. This diet contained

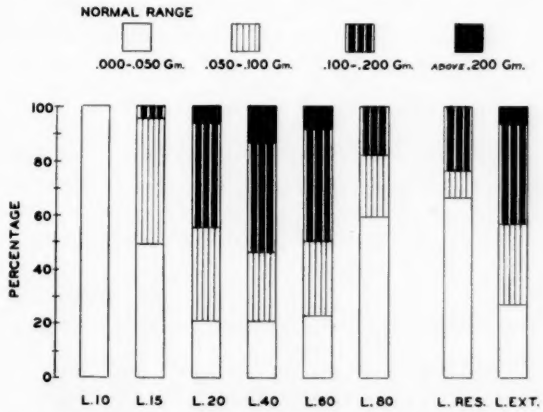

Chart 3.—The relation of urinary protein to diet in partially nephrectomized rats.

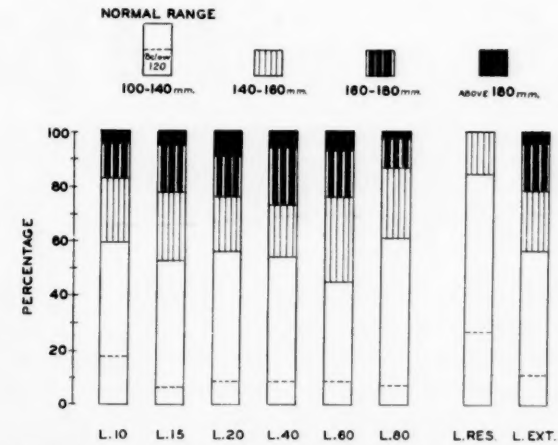

Chart 4.—The relation of blood pressure to diet in partially nephrectomized rats.

the highest percentage of protein, but it was free from the water-soluble liver substances. Strangely enough, the next lowest incidence of hypertension was in the group receiving diet L. 80, which was the group with the next highest concentration of protein and a large content of the water-soluble liver constitutents. This was more remark-

able when compared with the group receiving diet L. 60, which had the largest percentage of animals with hypertension. The differences between the other groups were not significant.

It is possible that the difference in time elapsed between the operation and the blood pressure readings in the different groups explains some of the apparent inconsistencies. It was found that hypertension did not appear in the group receiving diet L. 10 until the hundred and fifty-second day after the operation, whereas in the other groups (except in that receiving the L. Res. diet) it was noted during the first sixty days. Owing to the fact that the number of animals killed at definite intervals was not the same, no comparison can be made between the various groups. This is further complicated by the fact that daily variations for

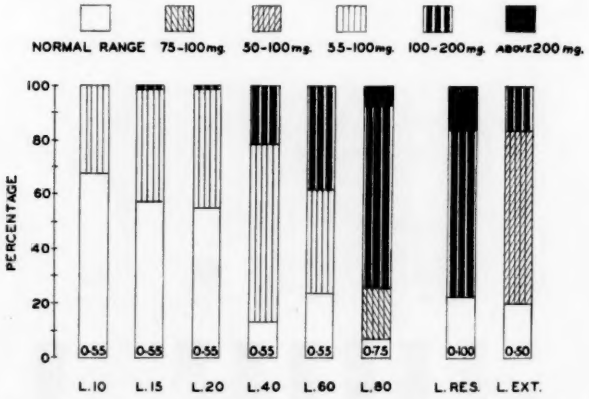

Chart 5.—The relation of azotemia to diet in partially nephrectomized rats. The normal range refers to values obtained in comparable control groups.

each rat could not be obtained, because only one reading for terminal blood pressure for each rat was possible.

Azotemia.—Chart 5 demonstrates the effect of the diets on the retention of nonprotein nitrogen. Significant retention of nitrogen was not noted in the groups receiving the L. 10, L. 15 and L. 20 diets. Additional increments of whole liver were followed by an increased incidence and degree of azotemia. The most marked retention was noted in the group receiving diet L. Res. The rats receiving the L. Ext. diet had a retention about equal to that of the group receiving diet L. 40. It was impossible to correlate the degree of retention of nitrogen with the health, renal function, albuminuria, blood pressure or pathologic changes (K'ang).

Heart.—In the majority of the animals the relationship between hypertension and the weight of the ventricles was the same as that found by Chanutin and Ferris [1] and Chanutin and Barksdale.[10] Most of the discrepancies noted were in the younger animals shortly after operation. The effect of diet on the weight of the ventricles seemed to be indirect, and dependent on the presence or absence of hypertension.

Liver.—The increased values of the $\frac{\text{L.W.}}{\text{S.A.}}$ ratio in the control animals receiving the L. 40, L. 60 and L. 80 diets were probably due to the high ingestion of protein and the deposition of fat from the relatively large amounts of cholesterol in the whole dried liver. Chemical analyses of a few representative animals from the groups receiving the L. 40, L. 60 and L. 80 diets showed abnormally large amounts of fat. This conforms with the findings of Blatherwick and his associates [11] of increased deposition of fat in the liver after the ingestion of diets rich in whole dried liver.

In striking contrast was the absence of this fatty change in the livers of the control group of animals receiving the L. Res. diet (highest in protein), though the values of the $\frac{\text{L.W.}}{\text{S.A.}}$ ratio were just as high as in the group receiving the L. 60 diet. Since the animals in this group were young at the beginning of the experiment, these observations are in accord with those of MacKay, MacKay and Addis,[12] who observed an increased weight of the liver in young rats fed a diet high in protein (casein).

The $\frac{\text{L.W.}}{\text{S.A.}}$ ratios of corresponding partially nephrectomized animals were of smaller value than those of the controls. It is difficult to attribute the smaller livers to renal insufficiency, particularly since the small livers of the animals receiving the diet containing whole liver were also fatty. The L. Ext. diet produced no significant change.

Kidney.—Chart 6 shows the effect of the diets on the $\frac{\text{K.W.}}{\text{S.A.}}$ ratio in the controls and the partially nephrectomized rats, the value of the ratio increasing with the nitrogen content of the diets containing whole liver and liver residue. Determinations of the total solids on a few of the kidney stumps indicated that a part, at least, of the increased weight was due to increased renal substance, a variable part being due to increased water.

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10. Chanutin, Alfred, and Barksdale, E. E.: Experimental Renal Insufficiency Produced by Partial Nephrectomy: II. Relationship of Left Ventricular Hypertrophy, the Width of the Cardiac Muscle Fiber and Hypertension in the Rat, Arch. Int. Med. **52**:739 (Nov.) 1933.

11. Blatherwick, N. R.; Medlar, E. M.; Bradshaw, Phoebe J.; Post, Anna L., and Sawyer, Susan D.: J. Biol. Chem. **103**:93, 1933.

12. MacKay, E. M.; MacKay, L. L., and Addis, T.: Am. J. Physiol. **86**:466, 1928.

MacKay and MacKay [13] showed that predictable increases in the weight of the kidney follow increases in protein in the diet, but that the increases could be materially lessened by adding enough vitamin B complex to the diet (MacKay [14]). Investigation showed that diets L. 20 to L. 80 contained adequate vitamin B complex to permit the successful reproduction of large healthy litters in the control animals, whereas diets L. 15 and L. 10 did not. The hypertrophy of the kidneys of animals receiving the L. Ext. diet was approximately what was to be expected from the nitrogen content. In these experiments the vitamin B complex, though sufficient to permit successful reproduction, did not prevent the progressive enlargement of the kidneys of the animals fed increasing amounts of whole liver.

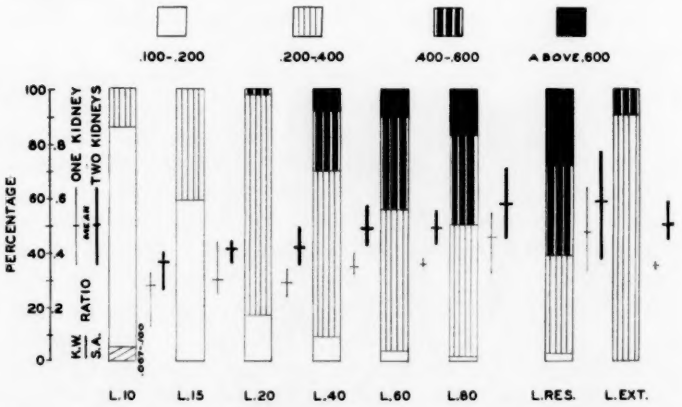

Chart 6.—The relation of $\frac{\text{K.W.}}{\text{S.A.}}$ ratio to diet in control and partially nephrectomized rats.

K'ang [9] found that the pathologic changes in the kidney stumps became more severe as the amount of whole dried liver in the diet was increased. The diet containing liver extract produced the most severe renal damage, and the diet containing liver residue the least.

Hemoglobin.—Hemoglobin determinations were not included in the tables because there was little difference among the animals fed the various diets. Whole liver or liver extract did not cause an excessive formation of hemoglobin, nor did it prevent moderate anemia in many. In a few rats acutely ill the anemia was rather severe. It was interesting that anemia could develop in animals receiving these liver diets, which were rich in the factors for preventing anemia.

13. MacKay, E. M., and MacKay, L. L.: J. Nutrition **3**:375, 1931.
14. MacKay, E. M.: Am. J. Physiol. **106**:571, 1933.

The Acute Condition in Partially Nephrectomized Rats.—Many animals became acutely ill within forty-eight hours after the second operation, when the intact kidney was removed. The course resembled that of animals after total nephrectomy. There was usual retention of nitrogen without edema and very low blood pressure.

Some animals became acutely ill after the first postoperative month, with listlessness, tetanic convulsions or quadrilateral paralysis with hypertension. They died in a few hours, some in coma, others in convulsions.

COMMENT

The relation between renal damage and hypertension is still unsolved. Among clinicians there is a difference of opinion as to whether renal insufficiency is a primary cause of hypertension. Following reduction of the functional renal substance in experimental animals pathologic changes occur which are accompanied by hypertension, cardiac hypertrophy, an increased flow of dilute urine, retention of nitrogen and the elimination of albumin and casts. In many respects this syndrome is analogous to certain types of renal involvement in man. The production of experimental hypertension is of importance, since it shows that hypertension can be secondary to renal damage.

In his discussion of renal insufficiency, Fishberg [15] stated:

> The height to which blood urea rises as a result of renal failure depends on various factors, notably the severity and duration of impairment of renal function, the fluid intake, the amount of protein in the diet, the rate of katabolism of protein in the body and perhaps the functional condition of the liver, the organ which forms urea. . . . There are no definite levels at which uremic symptoms set in or death occurs. Uremic coma occurs on rare occasions with less than 100 mg. per cent of urea in the blood, particularly if the protein content of the diet has been rigidly restricted and large quantities of carbohydrates successfully administered.

It is of interest to note that in partially nephrectomized rats, as in human beings with damaged kidneys, there is no exact relationship between the retention of nitrogen and morbid symptoms. Furthermore, it should be noted that in such rats the degree of retention is related to the nitrogen content of the diet.

Keutmann and McCann [16] reported that patients with chronic Bright's disease appeared to show a general improvement after ingesting large amounts of protein. These observations were conducted for a relatively short time, and the highest amounts of protein ingested comprised about 25 per cent of the total amount of energy of the diet. This

15. Fishberg, A. M.: Hypertension and Nephritis, ed. 3, Philadelphia, Lea & Febiger, 1934, p. 24.

16. Keutmann, E. H., and McCann, W. S.: J. Clin. Investigation **11**:973, 1932.

maximum intake of protein would correspond roughly to the experimental diet containing 40 per cent whole liver, in respect to the caloric value of the protein present. The larger concentrations of whole liver, liver residue and liver extract fed to rats probably could not be tolerated by man for any length of time.

It has been demonstrated in these experiments that a diet low in protein (L. 10) will delay, but will not prevent, the appearance of hypertension; in addition, there is little retention of nitrogen, albuminuria and polyuria. These findings may serve as an experimental confirmation for the use of diets low in protein in renal damage or hypertension in man. Relatively small amounts of protein in the diets that were considered to be normal for intact rats (L. 15 and L. 20) were responsible for an increased excretion of dilute urine containing more urinary protein and for the early appearance of hypertension. In contrast to these observations it was seen that the experimental animals ingesting a diet extremely rich in protein (L. Res.) showed no marked effect on the blood pressure, albuminuria or renal changes despite the increased excretion of dilute urine and the marked retention.

The conflicting results seen in clinical observations have been encountered in the present experiments. An effort to find a common factor which could be correlated with the various experimental findings has not been successful. Moderate and severe hypertension were encountered with and without marked renal insufficiency. Furthermore, the degree of hypertension was not dependent on the extent of renal damage as judged by the concentration test, retention of nitrogen or pathologic changes. The amount of urinary protein excreted was not predictable from the quantity of urine excreted. Apparently renal insufficiency alone cannot be the direct cause of the findings noted in this study.

SUMMARY

The effect of feeding diets containing varying percentages (10, 15, 20, 40, 60 and 80) of whole dried liver, liver residue (80) and aqueous liver extract to intact, unilaterally nephrectomized and partially nephrectomized rats has been studied.

The ingestion of these diets by intact and unilaterally nephrectomized animals was followed by enlargement of the kidneys which was roughly proportional to the nitrogen content of the diet; the liver was similarly increased in weight owing to the deposition of fat and the effect of ingestion of an increased amount of protein; the azotemia was more marked in those animals receiving the diets containing more liver; the renal function was unaffected. An appreciable number of unilaterally nephrectomized rats had slightly increased blood pressure.

The syndrome obtained in the partially nephrectomized rats was affected by the percentage of whole liver and liver fractions in the diet in the following manner:

1. A progressively increased quantity of dilute urine was excreted as the content of whole liver was raised. Neither the administration of liver residue nor of liver extract caused marked changes in the volume or specific gravity of the urine. The amount of urinary protein excreted had no relation to the volume of urine.

2. Hypertension was delayed in the rats receiving the diet that is 10 per cent whole liver. The addition of a small amount of liver had a definite effect on the early appearance of hypertension. The rats receiving the diet containing liver residue showed practically no hypertension despite evidence of renal damage.

The degree of azotemia appeared to depend on the amount of nitrogen in the diet.

In the older animals there was a relationship between the blood pressure and the $\frac{\text{H.W.}}{\text{S.A.}}$ ratio. Discrepancies in this relationship were noted in the younger animals. The values of the $\frac{\text{L.W.}}{\text{S.A.}}$ ratio were smaller than those for the corresponding controls. The values of the $\frac{\text{K.W.}}{\text{S.A.}}$ ratio were extremely variable, but were roughly proportional to the amount of nitrogen in the diet.

In partially nephrectomized animals, the degree of renal insufficiency, albuminuria, retention of nitrogen and, to a certain extent, the occurrence and degree of hypertension were found to vary according to the diet fed. The factors responsible for the acute illness, hypertrophy of the kidney, polyuria and hypertension are still unknown and poorly understood.

THERAPEUTIC EFFECT OF TOTAL ABLATION OF NORMAL THYROID ON CONGESTIVE HEART FAILURE AND ANGINA PECTORIS

VIII. RELATIONSHIP BETWEEN SERUM CHOLESTEROL VALUES, BASAL METABOLIC RATE AND CLINICAL ASPECTS OF HYPOTHYROIDISM

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The relationships between the serum cholesterol concentration, the basal metabolic rate and the clinical aspects of hypothyroidism induced by total removal of the normal thyroid gland in patients with chronic intractable heart disease have been studied and are reported in this communication.[1] By means of preoperative measurements and measurements at appropriate intervals after total thyroidectomy, it has been possible to observe the development of changes in the serum cholesterol values and the basal metabolic rate as hypothyroidism developed in these patients who before operation had no thyroid disease. The development of untoward symptoms of myxedema necessitated the administration of minimal amounts of thyroid substance to many of the patients, thus affording an opportunity to study the effect of this medication on the aforementioned relationships.

Mason, Hunt and Hurxthal[2] reviewed the literature up to 1930 on the relationship of blood cholesterol concentration to the activity of the thyroid gland in thyroid disease. Further studies by these authors strengthen the observation that the plasma cholesterol is increased in

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1. The operations were performed by Dr. David D. Berlin and Dr. Charles G. Mixter.

2. Mason, R. L.; Hunt, H. M., and Hurxthal, L. M.: Blood Cholesterol Values in Hyperthyroidism and Hypothyroidism: Their Significance, New England J. Med. **203**:1273, 1930.

myxedema and, less regularly, decreased in thyrotoxicosis. No exact quantitative relationship, however, was found between the plasma cholesterol concentration and the basal metabolic rate. When, by appropriate therapeutic procedures, the basal metabolic rate in myxedema and hyperthyroidism is rendered normal, the abnormal plasma cholesterol concentration likewise returns to normal.[3] The plasma cholesterol concentration, therefore, has been employed as an aid in the diagnosis and treatment of thyroid disease.[4] Especially in cases in which reliable measurements of basal metabolic rates cannot be obtained, the plasma cholesterol concentration has been found to be of distinct value.[3a]

METHODS

Blood for cholesterol measurements was drawn from the antecubital vein, with minimal stasis, after a fast of fourteen hours or more. Measurements of serum cholesterol were made in duplicate by the method of Myers and Wardell,[5] using the apparatus for continuous extraction described by Ling.[6] The averages of the duplicate measurements, which checked within 5 per cent, have been reported. The high limit accepted for normal serum cholesterol values is generally about 230 mg. per hundred cubic centimeters.

Measurements of the basal metabolic rate were made in duplicate with a Collins-Benedict-Roth apparatus, and results calculated according to the Aub-DuBois normal standards.[7] The averages of duplicate analyses, which checked within 5 per cent, are reported. The preoperative values represent the average of several such measurements on different days. Preoperative measurements and those taken during the first two postoperative weeks were made while the patient was hospitalized. The majority of the basal metabolic rates after the first two weeks were measured when the patient came to the laboratory from his home and rested quietly in bed for from one-half to one hour. Although the latter condition is not as ideal as when patients stay overnight in the hospital, it is felt the measurements of the basal metabolic rate given here are reliable.

When weakness of the legs, drowsiness, emotional irritability or puffiness of the face developed, thyroid substance was administered in appropriate amounts to alleviate these untoward symptoms of myxedema without raising the basal metabolic rate sufficiently to place an undue burden on the heart. The amounts of

3. (*a*) Bronstein, I. P.: Studies in Cretinism and Hypothyroidism in Childhood: I. Blood Cholesterol, J. A. M. A. **100**:1661 (May 27) 1933. (*b*) Hurxthal, L. M.: Blood Cholesterol in Thyroid Disease: II. Effect of Treatment, Arch. Int. Med. **52**:86 (July) 1933. (*c*) Levy, M., and Levy, M. (Mme): Le traitement de l'hypercholestérinemie par la thyroxine, Bull. Acad. de méd., Paris **105**:666, 1931. (*d*) Mason, Hunt and Hurxthal.[2]

4. Mason, Hunt and Hurxthal.[2] Bronstein.[3a] Hurxthal.[3b]

5. Myers, V. C., and Wardell, E. L.: The Colorimetric Estimation of Cholesterol in Blood, with a Note on the Estimation of Coprosterol in Feces, J. Biol. Chem. **36**:147, 1918.

6. Ling, S. M.: The Determination of Cholesterol in Small Amounts of Blood, J. Biol. Chem. **76**:361, 1928.

7. Aub, J. C., and Du Bois, E. F.: Clinical Calorimetry: XIX. The Basal Metabolism of Old Men, Arch. Int. Med. **19**:823 (May) 1917.

thyroid substance administered were so adjusted as to maintain the patient in a hypothyroid state, as evidenced by dry skin, increased sensitivity to cold and a low basal metabolic rate.

RESULTS

Simultaneous measurements of the serum cholesterol concentration and the basal metabolic rate were made in twenty-nine patients before and at frequent intervals after complete thyroidectomy; in eighteen other patients the same measurements were made at postoperative intervals only (table). The ages of the subjects studied varied from 14 to 66 years; sixteen were female and thirty-one male. Twenty-eight patients suffered from congestive heart failure at the time of or just prior to operation, fourteen had angina pectoris and five had no cardiac involvement.

Relationships Between Serum Cholesterol Value and Basal Metabolic Rate Before Total Thyroidectomy.—The preoperative serum cholesterol values ranged between 92 and 298 mg. per hundred cubic centimeters, and the basal metabolic rates in the same twenty-nine cases varied between plus 13 and minus 24 per cent (table). Seven subjects had preoperative serum cholesterol values above 230 mg. per hundred cubic centimeters. Four of these had coexisting diseases, such as diabetes (cases 23, 25 and 27) and Paget's disease (case 28), which may increase the serum cholesterol concentration.[8] The other three patients with high preoperative serum cholesterol values had basal metabolic rates between minus 14 and minus 23 per cent (cases 24, 26 and 29). Although one cannot detect any further significant correlation between the basal metabolic rate and the serum cholesterol concentration in the subjects of this study, a tendency toward such a correlation has been observed by studying a much larger group of subjects with no disease of the thyroid gland.[9]

Relationship Between Serum Cholesterol Concentration and Basal Metabolic Rate in Untreated Hypothyroidism After Total Thyroidectomy.—As the basal metabolic rate decreased, the serum cholesterol concentration increased, following total ablation of the thyroid gland (table). In a given patient the increase in serum cholesterol concentration bore no precise quantitative relation to the decrease in basal metabolic rate. The highest cholesterol values obtained were, however, found in six of the eight patients whose basal metabolic rates were decreased to minus 35 per cent or lower. In one patient (case 39) the basal metabolic rate had not shown a significant decrease by the end

8. Lasch, F.: Biochemische Untersuchungen bei Ostitis deformans Paget, Wien. Arch. f. inn. Med. **21**:159, 1931.

9. Volk, M. C.; Abrams, M. I., and Gilligan, D. R.: The Relationship Between Serum Cholesterol and Basal Metabolism in Subjects with no Thyroid Disease, With Particular Reference to Subjects with Low Basal Metabolic Rates, to be published.

of the second postoperative month. The serum cholesterol concentration at this time, however, was increased to 368 mg. per hundred cubic centimeters, and clinical evidences of hypothyroidism were present.

A change in both the basal metabolic rate and the serum cholesterol concentration was usually observed as early as the first postoperative week. By the end of the first postoperative month the basal metabolic rate had usually decreased to approximately its lowest value, whereas the serum cholesterol concentration tended to increase steadily during the next few months (figure). Thus, in a series of patients who were not receiving thyroid medication, the average basal metabolic rate fluctuated between the narrow limits of from 25 to 29 per cent below the preoperative average during the period from one to six months after operation, whereas the average serum cholesterol value gradually increased during this period from 88 to 153 mg. per hundred cubic centimeters above the preoperative average.

Relationship of the Serum Cholesterol and the Basal Metabolic Rate to Untreated Signs and Symptoms of Myxedema After Total Thyroidectomy.—After varying periods of time some of the signs and symptoms of myxedema developed in every patient following total thyroidectomy. By the end of the first postoperative month most of the patients showed mild clinical signs and symptoms, such as dry skin, slight pallor and sensitivity to cold. After the second postoperative month more serious symptoms of myxedema developed in many of the patients, such as weakness of the extremities, drowsiness, emotional irritability and transient puffiness of the face, hands and feet. The serious symptoms were relieved by the administration of small doses of thyroid substance.

When the first clinical signs and symptoms of myxedema appeared, the basal metabolic rate was usually reduced to minus 20 per cent or lower, and the serum cholesterol value was, with few exceptions, above 200 mg. per hundred cubic centimeters (table). The untoward symptoms of myxedema were usually not observed until the basal metabolic rate had remained at levels of approximately minus 30 per cent from a few weeks to months, and the serum cholesterol value had increased to approximately 300 mg. per hundred cubic centimeters or higher. However, in six of the patients (cases 1, 13, 21, 27, 29 and 41) distressing symptoms of myxedema were manifest when the measurements of the basal metabolic rate gave values between minus 12 and minus 26 per cent. In these six instances the serum cholesterol was increased, the values ranging between 283 and 482 mg. per hundred cubic centimeters, five patients showing concentrations above 300 mg. per hundred cubic centimeters.

Three patients (cases 7, 16 and 36) operated on from ten to fifteen months ago have not as yet required thyroid medication for the alleviation of untoward symptoms of myxedema. The basal metabolic

Serum Cholesterol Concentrations and Basal Metabolic Rates Before and at Varying Intervals After Total Ablation of the Normal Thyroid Gland

				Before Operation		After Total Thyroidectomy																	
						1 Week		2 Weeks		1 Month		2 Months		3 Months		4 Months		5 to 6 Months		9 to 10 Months		12 Months		
Case	Age	Sex	Diagnosis*	B. M. R.: Deviation from Normal, per Cent	Serum Cholesterol, Mg. per 100 Cc.	B. M. R.: Deviation from Normal, per Cent	Serum Cholesterol, Mg. per 100 Cc.	B. M. R.: Deviation from Normal, per Cent	Serum Cholesterol, Mg. per 100 Cc.	B. M. R.: Deviation from Normal, per Cent	Serum Cholesterol, Mg. per 100 Cc.	B. M. R.: Deviation from Normal, per Cent	Serum Cholesterol, Mg. per 100 Cc.	B. M. R.: Deviation from Normal, per Cent	Serum Cholesterol, Mg. per 100 Cc.	B. M. R.: Deviation from Normal, per Cent	Serum Cholesterol, Mg. per 100 Cc.	B. M. R.: Deviation from Normal, per Cent	Serum Cholesterol, Mg. per 100 Cc.	B. M. R.: Deviation from Normal, per Cent	Serum Cholesterol, Mg. per 100 Cc.	B. M. R.: Deviation from Normal, per Cent	Serum Cholesterol, Mg. per 100 Cc.	Thyroid Medication, Daily Dosage
1. W. D.	22	M	C.H.F.	— 8	92	....	...	—16	...	—32	140	—32	152	—27	...	—26	...	—24	283†	—26	204	—28	260	½ to ⅓ gr.
2. L. M.	28	M	C.H.F.	— 2	96	....	261	—18	315															
3. A. R.	50	M	N.C.	— 2	101	....	...	—20	...	—34	238													
4. R. D.	22	M	C.H.F.	— 3	109	—24	...	—22	...	—37	...†	—30	203	—25	171	—27	...	....	...	....	...	....	...	1 to ½ gr.
5. W. B.	63	M	C.H.F.	—10	117	....	...	—21	...	—31	191	—29	...	—41	428‡	....	...	....	...†	....	...	....	...	¼ gr.
6. M. W.	54	M	A.P.	— 7	119	....	...	—12	...	—16	284	—20	...	—30	...†	—30	278	—34	202	....	...	....	...	¼ to ⅓ gr.
7. E. W.	27	M	C.H.F.	+ 5	127	....	...	+ 5	...	—19	135	—22	...	—27	149	....	...	—34	181					
8. L. B.	44	F	C.H.F.	— 9	132	— 3	...	—22	...	—32	...	—25	310‡	....	...	....	...	—37	385†§	—32	400	....	...	¼ to ⅛ gr.
9. F. Z.	21	M	C.H.F.	+ 1	138	— 3	...	— 8	199	....	...	....	...	....	...	—10	254	—18	246	—24				
10. H. G.	22	M	C.H.F.	+ 7	138	....	...	—21	204	....	...	—28	...	—27	251†	—22	194	—38	236	—30	...	....	...	¼ gr.
11. B. C.	35	F	C.H.F.	— 1	145	—12	154	....	...	—33	280	—15	317	—27	...†	....	...	—19	294	....	...	....	...	¼ gr.
12. F. C.	35	M	C.H.F.	— 4	154	—13	...	—24	...	—24	...	—32	217	....	...	....	...	—27	255†	—37	216	—29	...	⅓ to ⅛ gr.
13. S. B.	51	F	C.H.F.	+ 4	154	....	214	—11	...	—12	311‡	....	...†	—23	...	—14	246	....	...	....	...	—16	...	¼ to 1/10 gr.
14. C. C.	66	M	C.H.F.	+ 9	154	—22	...	—23	147	—36	207†	—15	196	—10	...	....	...	—30	206	....	...	....	...	⅓ to ⅓ gr.
15. S. B.	47	F	C.H.F.	+13	161	+12	...	....	...	— 6	...	....	364	....	...	....	375	—11	...†	....	...	....	...	⅛ gr.
16. B. Z.	45	F	C.H.F.	— 5	167	....	...	—16	...	—30	214	—27	...	—27	250	....	...	—20	408	—21	448	—16	397	
17. W. M.	63	M	N.C.	+ 1	168	....	...	—11	...	—21	...	—33	320‡	—31	305	—30	...†	—33	...	—25	...	—27	...	¼ gr.
18. F. D.	18	M	C.H.F.	—12	172	....	...	—28	220	—21	...	—26	...	—36	323†	....	...	—34	294	....	...	—30	...	¼ gr.
19. F. F.	52	M	N.C.	0	180	....	...	+ 1	...	—15	...	—25	180†	....	189	—25	...	....	...	....	...	....	...	½ gr., 1 mo.
20. M. C.	57	M	A.P.	—24	192	....	...	—26	...	—34	...	—34	347†	—32	435	—35	381	—32	284	....	...	....	...	½ to ¼ gr.

21. L. M.	55	M	C.H.F.	+ 6	200	—12	232	....	...	—34	...	....	...	—21	345	....	...	—26	400†	—28	...§	—32	...	¼ gr.
22. A. B.	59	M	A.P.	— 4	211	....	...	—16	220	—28	...	—24	...	—30	298†	....	...	—31	315	—20	205	....	...	⅛ to ⅙ gr.
23. M. V.	59	F	A.P., D.	—13	231	—17	...	....	...	—22	...	....	...	—33	...	....	...	—26	343†	—18	...	....	...	⅛ gr.
24. M. P.	53	M	A.P.	—15	240	—14	311†	—25	...	—30	...	—32	446‡	—37	...†	—28	...	—34	...	....	...	....	...	¼ gr.
25. G. M.	45	F	C.H.F., D.G.T.	— 6	250	—11	192	—10	240	—24	304	....	...†	....	...	....	...	....	...	—27	...	....	...	¼ to ⅛ gr.
26. M. F.	48	M	A.P.	—14	251	....	...	—17	...	—16	...	—33	450‡	....	...	—30	...†	....	...	—30	...	....	...	⅛ to ⅛ gr.
27. R. S.	57	F	A.P., D.	+ 3	292	....	...	+ 3	...	....	...	—19	399‡	—26	...	....	...	....	...	—23	...	—23	...†	3/10 gr.
28. W. B.	55	M	C.H.F., P.D.	+ 9	294	— 2	...	—15	335	—16	...	—24	388†	—22	...	....	...	—33	410	....	...	—27	500	⅛ gr., 1 mo.
29. E. P.	58	F	A.P.	—23	298	....	...	—20	...	—23	...	—21	482†	—20	435§	....	...	—31	543†	—25	...	—25	...	1 to ¼ gr.
30. R. M.	14	M	N.C.	....	180	....	...	....	...	....	248	—47	394‡	—42	...†	....	...	....	...	....	...	....	...	⅛ gr.
31. H. G.	43	F	C.H.F.	— 7	...	....	...	—19	...	....	...†	....	202	—36	345	....	...	—33	521	—28	...	....	...	½ to ⅕ gr.
32. T. C.	64	M	A.P.	—19	...	—19	...	—27	182	—38	373†	—34	319	—33	...	—35	...	—31	...	....	...	....	...	¼ gr.
33. M. S.	46	M	A.P.	— 8	...	—21	282	—20	333	—20	...	....	...†	—25	...	....	...	....	...	....	...	....	...	¼ gr.
34. H. K.	60	M	A.P.	—17	...	—29	189	—37	231†	—22	278	—30	...	....	...	....	...	—34	...	—31	...	....	...	¼ to ⅛ gr.
35. S. M.	48	M	A.P.	+ 7	...	....	...	—34	357	—27	...†	....	...	....	...	....	...	....	...	....	...	....	...	¼ gr.
36. G. F.	52	M	C.H.F.	— 2	...	....	...	—14	...	—23	...	—31	...	—26	...	—28	...	—25	286	—26	...	—26	303	
37. M. A.	19	F	N.C.	— 7	...	....	...	—17	...	—36	...	—41	400†	— 2	269	—18	...	....	...	—22	...	....	...	¼ to ⅛ gr.
38. S. F.	52	M	A.P.	....	...	—28	162	—24	...	—37	234	—31	...	—43	360†	....	...	—20	149	—29	...	....	...	1 to ⅛ gr.
39. B. K.	50	F	C.H.F.	+14	...	....	...	+ 6	...	+12	237	+ 7	368	....	...	....	520‡							
40. H. B.	27	F	C.H.F.	— 9	...	—11	...	....	...	—21	263	—23	...	—30										
41. B. R.	48	F	C.H.F.	+ 3	...	....	...	—16	294	—10	325	—14	404†	—15	318	—21	250	—12	257	....	...	....	...	⅛ gr.
42. G. O.	65	M	A.P.	—13	...	....	...	....	197	—28	322	—34	301	—28	...	....	...	—30	280†	—35	...	....	...	⅛ gr.
43. J. R.	63	M	C.H.F.	+24	...	....	...	+ 3	...	....	...	—22	328	—28	424†	....	...	—26	...	....	...	....	...	⅛ gr.
44. M. G.	39	F	C.H.F.	+ 3	...	....	...	—18	...	— 3	...	—15	400†	—12	295	....	...	—14	...	....	...	....	...	¼ gr.
45. E. M.	46	F	C.H.F.	— 4	...	—24	...	....	...	....	...	—23	366‡	—34	...†	....	...	....	...	—23	...	....	...	⅛ gr.
46. J. T.	59	M	C.H.F.	— 3	...	....	...	—10	...	—31	471	—28	...†	—21	330	....	...	—20	330	....	...	....	...	¼ to ⅕ gr.
47. M. H.	54	M	A.P.	+ 5	...	+ 3	148	— 4	184	....	333†	....	342	....	...	....	...	—28	...	....	323	....	...	½ to ⅛ gr.

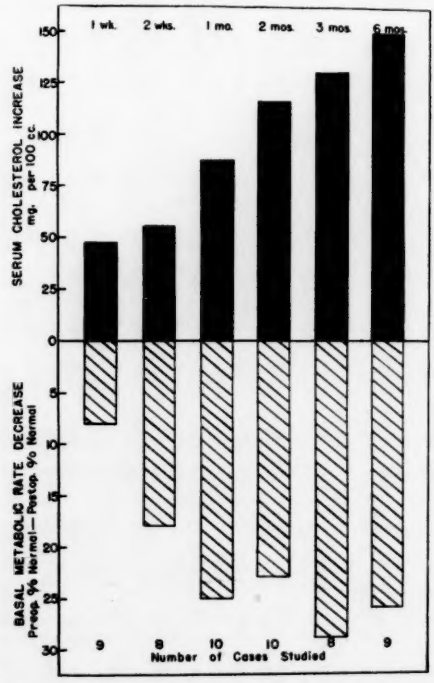
* In the list of conditions diagnosed, C.H.F. indicates congestive heart failure; N.C., noncardiac condition; A.P., angina pectoris; D., diabetes; P.D., Paget's disease, and D.G.T., dextrose tolerance curve of diabetic type.

† Thyroid medication instituted at the time of the appearance of untoward symptoms of myxedema.

‡ Appearance of symptoms of advanced myxedema; thyroid indication withheld for observation.

§ Thyroid medication discontinued for study or against advice.

rates in these patients have been persistently low (table). In two of them the highest serum cholesterol values obtained at any time since operation were 181 and 303 mg. per hundred cubic centimeters (cases 7 and 36) and in the third (case 16) 450 mg. The latter patient was the only one who maintained a cholesterol level significantly above 300 mg per hundred cubic centimeters for many months, in the absence of a concomitant disease associated with a high serum cholesterol level, and



Relationship between the average changes in the serum cholesterol concentration and average changes in the basal metabolic rate following total thyroidectomy. The values presented were obtained in patients on whom both measurements were made preoperatively, and who after operation had not yet received thyroid medication. In two of the six patients whose cholesterol concentrations were measured one week after operation, the basal metabolic rates for the period were obtained by interpolation from the values obtained preoperatively and two weeks postoperatively. The average preoperative basal metabolic rate for all cases represented was minus 4 per cent, and the average serum cholesterol value was 174 mg. per hundred cubic centimeters.

yet did not require thyroid medication for the control of untoward symptoms of myxedema.

Since the untoward symptoms of myxedema usually developed when the level of the metabolic rate was maintained significantly below minus 30 per cent, the extent of decrease in the basal metabolic rate which could be induced in a given patient by total thyroidectomy was proportional to the height of the preoperative basal metabolic rate (table). To cite two extreme instances from our series, the patient in case 20, whose preoperative basal metabolic rate was minus 24 per cent, required thyroid medication after a further decrease in the basal metabolic rate of only 10 per cent (minus 34), and the patient in case 14, whose preoperative basal metabolic rate was plus 9 per cent, required thyroid medication after a net decrease in the basal metabolic rate of 45 per cent (minus 36).

Effect of Small Doses of Thyroid Medication on the Serum Cholesterol, the Basal Metabolic Rate and the Clinical Signs and Symptoms of Myxedema.—Since thyroidectomy was performed in patients with chronic heart disease for the therapeutic purpose of inducing hypothyroidism, according to the considerations outlined elsewhere,[10] the mild signs and symptoms of myxedema were allowed to persist in order to maintain the basal metabolic rate at a low level. Small amounts of thyroid substance were administered when the untoward symptoms of myxedema developed. The amount of thyroid substance required to control the symptoms and at the same time to maintain the basal metabolic rate at a low level varied between ⅛ and ¼ grain (8.1 and 32.4 mg.) a day, the dosage being adjusted in each patient by trial. When the optimum amount of thyroid was administered, significant decreases in the serum cholesterol concentrations resulted (table). Thyroid medication was usually indicated between the end of the first and the sixth postoperative months, the period during which the serum cholesterol concentrations showed progressive increases, although the basal metabolic rates showed minor or no decreases (figure).

10. Blumgart, H. L.; Levine, S. A., and Berlin, D. D.: Congestive Heart Failure and Angina Pectoris: The Therapeutic Effect of Thyroidectomy on Patients Without Clinical or Pathologic Evidence of Thyroid Toxicity, Arch. Int. Med. **51**:866 (June) 1933. Berlin, D. D.: Therapeutic Effect of Complete Thyroidectomy on Congestive Heart Failure and Angina Pectoris in Patients with no Clinical or Pathological Evidence of Thyroid Toxicity: II. Operative Technique, Am. J. Surg. **21**:173, 1933. Blumgart, H. L.; Riseman, J. E. F.; Davis, D., and Berlin, D. D.: Therapeutic Effect of Total Ablation of Normal Thyroid on Congestive Heart Failure and Angina Pectoris: III. Early Results in Various Types of Cardiovascular Disease and Coincident Pathologic States Without Clinical or Pathologic Evidence of Thyroid Toxicity, Arch. Int. Med. **52**:165 (Aug.) 1933.

COMMENT

Blumgart and his associates have demonstrated [11] that patients with chronic heart disease and no hyperthyroidism are benefited by the decreased burden on the heart which obtains in hypothyroidism induced by total ablation of the thyroid gland. The changes in the basal metabolic rate and the serum cholesterol which occur after operation together with the relationship of these changes to the clinical aspects of hypothyroidism are reported here.

In the group of twenty-nine patients in whom the basal metabolic rates and cholesterol concentrations were measured before operation (table) twenty-five had basal metabolic rates between plus 9 and minus 13 per cent. There was no evident correlation between the serum cholesterol concentration and the basal metabolic rate before operation. However, in the other four (cases 20, 24, 26 and 29), all of whom suffered from angina pectoris, the basal metabolic rates ranged between minus 14 and minus 24 per cent, and in three of these the serum cholesterol values were between 240 and 298 mg. per hundred cubic centimeters. It is believed, in retrospect, that signs suggestive of myxedema were present before operation in one of them (case 29). In the other three patients there were no clinical evidences of decreased thyroid activity before operation. Pathologic studies of the thyroid glands from the four patients were made by Dr. Monroe Schlesinger, and revealed normal observations.

In a recent investigation,[9] studies of the basal metabolic rate and serum cholesterol values in a group of normal subjects showed that persons with no clinical evidence of hypothyroidism may have basal metabolic rates as low as from minus 20 to minus 35 per cent, and that the serum cholesterol concentration in such persons may be increased to between 230 and 290 mg. per hundred cubic centimeters.

The basal metabolic rate decreased following total thyroidectomy, and remained persistently low in forty-seven of the forty-eight patients studied in this series. Basal metabolic rates as low as from minus 41 to minus 47 per cent were observed postoperatively in four. Such levels could not be maintained, because thyroid medication was required to control untoward symptoms of myxedema. Although it was impossible to measure the lowest levels of metabolic rate which patients deprived of all thyroid tissue might have attained if allowed to continue without thyroid medication, it is believed that the levels from minus 41 to minus 47 per cent, which were attained after the end of the first postoperative month, represent the approximate metabolic levels of completely thyroprival persons. This observation is in accord

11. Friedman, H. F., and Blumgart, H. L.: Treatment of Chronic Heart Disease by Lowering the Metabolic Rate: The Necessity for Total Ablation of the Thyroid, J. A. M. A. **102**:17 (Jan. 6) 1934. Footnote 10.

with the concept of Means [12] and others that the normal thyroid activity controls from 40 to 45 per cent of the caloric requirements of the human subject, and with the fact that basal metabolic rates lower than minus 47 per cent of normal [2] are rarely if ever encountered in hypothyroidism, either of idiopathic or postoperative origin.

Decreases in the basal metabolic rate following total thyroidectomy were accompanied by increases in the serum cholesterol concentration, the highest cholesterol value obtained being 543 mg. per hundred cubic centimeters, in a patient who showed marked symptoms of myxedema at the time of the test. The serum cholesterol values obtained in the patients with postoperative hypothyroidism were similar to those obtained by other investigators in patients with spontaneous myxedema. Thus, Mason, Hunt and Hurxthal [2] reported cholesterol values as high as 500 mg. per hundred cubic centimeters associated with markedly lowered basal metabolic rates in patients with spontaneous myxedema, and Bronstein [3a] reported a blood cholesterol value of 782 mg. per hundred cubic centimeters in a child with hypothyroidism. The highest serum cholesterol values are obtained in those patients who show the most advanced clinical symptoms [13] (table). When the more serious symptoms of myxedema were controlled by appropriate thyroid medication, the serum cholesterol value was generally maintained at a level of about 300 mg. per hundred cubic centimeters.

The patient in case 39 (table) is the only one in a consecutive series of seventy operated on at this hospital who did not show a low basal metabolic rate following total thyroidectomy. Measurements of the basal metabolic rate in this case gave values of plus 14 per cent preoperatively and plus 12 and plus 7 per cent at the end of the first and second postoperative months, respectively. A measurement at the end of the fourth postoperative month was unsatisfactory. The serum cholesterol value one month after operation was 237 mg. per hundred cubic centimeters and showed further marked increases to 368 and 520 mg. at the end of the second and fourth postoperative months, respectively. The progressive increase in serum cholesterol values together with clinical manifestations of myxedema in this patient offered evidence that a state of hypothyroidism had been induced by the operation and that the persistence of a basal metabolic rate at a normal level may have been due to inability to obtain a basal test.

In six patients of this series the untoward symptoms of myxedema, such as weakness of the legs, drowsiness, irritability and puffiness of the face, hands and feet, were manifest when the basal metabolic rate was reduced only from minus 12 to minus 26 per cent. In four of the six, the serum cholesterol concentrations were increased above

12. Means, J. H.: Personal communication to the authors.

13. Mason, Hunt and Hurxthal.[2] Bronstein.[3a]

300 mg. per hundred cubic centimeters. Although basal metabolic rates similarly reduced are encountered in normal persons, serum cholesterol values of this magnitude are not obtained.[14] These considerations suggest that true measurements of the basal metabolism may not have been obtained in some of the six with untoward symptoms of myxedema. This suggestion is strengthened by the fact that some of the patients had shown lower basal metabolic rates earlier in the postoperative course, before the development of the advanced symptoms of myxedema.

On several occasions we have been unable to obtain duplicate measurements of the basal metabolic rate which agreed within 5 per cent. In these instances the serum cholesterol measurements have been a useful adjunct to clinical observations.

That the serum cholesterol concentration may give evidence of hypothyroidism when the basal metabolic rate does not is shown by observations on patients with myxedema after the administration of *di*-nitro-phenol and *di*-nitro-*ortho*-cresol. When the basal metabolic rate is increased to or above normal by these drugs in patients with myxedema, the clinical signs and symptoms and the high serum cholesterol concentration persist unchanged.[15]

There seems to be a general belief that myxedema and hypercholesteremia predispose one to arteriosclerosis. There is little evidence from observations in man, however, to support these theories.[16] Roentgenologic studies of the blood vessels in six patients in this group who had hypothyroidism and associated hypercholesteremia of a year's duration showed no apparent increased density.

Since the level of the basal metabolic rate at which the untoward symptoms of myxedema develop is usually about minus 30 per cent, only a moderate reduction in the basal metabolic rate after total thyroidectomy can be effected in those patients who before operation have lowered basal metabolic rates[17] (table).

Measurements of serum cholesterol concentration have served as an adjunct to clinical impressions in the evaluation and control of the

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14. Mason, Hunt and Hurxthal.[2] Volk, Abrams and Gilligan.[9]

15. Dodds, E. C., and Robertson, J. D.: Clinical Applications of Dinitro-Cresol, Lancet **2**:1137 and 1197, 1933. Rabinowitch, I. M., and Fowler, A. F.: Dinitrophenol, Canad. M. A. J. **30**:128, 1934.

16. Means, J. H., and Richardson, E. P.: The Diagnosis and Treatment of Diseases of the Thyroid, in Christian, H. A.: Oxford Monographs on Diagnosis and Treatment, New York, Oxford University Press, 1929, vol. 4. Peters, J. P., and Van Slyke, D. D.: Quantitative Clinical Chemistry, Baltimore, Williams & Wilkins Company, 1931, vol. 1, p. 238.

17. Blumgart, H. L.; Davis, D.; Riseman, J. E. F.; Berlin, D. D., and Weinstein, A. A.: Treatment of Angina Pectoris and Congestive Failure by Total Ablation of the Thyroid: XI. Résumé of Results in Treating Seventy-Five Patients During the Last Eighteen Months, J. A. M. A., to be published.

hypothyroid state which develops following total thyroidectomy. Since the value of the measurement does not depend on the cooperation of the patient, it has proved at times to be a useful laboratory test in determining the presence or degree of hypothyroidism in patients who are unable to obtain the relaxation necessary for measurement of the true basal metabolic rate. The measurement of serum cholesterol is also of considerable value as an adjunct to clinical impressions in the estimation of the degree and course of hypothyroidism when, during the development of the hypothyroid state, the basal metabolic rate has reached and is maintained at a low level.

SUMMARY

1. Simultaneous measurements of the serum cholesterol concentration and basal metabolic rate have been made in a group of patients before and at intervals during the development of the hypothyroid state after total ablation of the normal thyroid gland.

2. Following total thyroidectomy, the serum cholesterol value increases as the basal metabolic rate decreases and as the clinical evidences of hypothyroidism become manifest. The highest serum cholesterol concentration obtained in any patient was 543 mg. per hundred cubic centimeters and the lowest basal metabolic rate was minus 47 per cent.

3. Appreciable changes in the basal metabolic rate and serum cholesterol concentration were generally evident by the end of the first postoperative week. Striking changes in the values were observed by the end of the first postoperative month. During the following few months the basal metabolic rate showed only minor, if any, decreases, whereas the serum cholesterol concentration gradually increased and the clinical course of hypothyroidism gradually progressed.

4. When the untoward symptoms of myxedema became manifest, the serum cholesterol concentration was usually increased to 300 mg. per hundred cubic centimeters or above, and the basal metabolic rate was usually decreased to minus 30 per cent or below.

5. Small doses of thyroid medication, administered to control the untoward symptoms of myxedema, caused slight increases in the basal metabolic rate and significant decreases in the serum cholesterol concentration.

6. In a few instances a high serum cholesterol value confirmed the clinical finding of untoward symptoms of myxedema when the basal metabolic rate was not markedly low. In other instances, when duplicate measurements of the basal metabolic rate which agreed within 5 per cent could not be obtained, the serum cholesterol concentration was a valuable adjunct to clinical impressions in estimating the degree and course of the hypothyroid state.

A RAPID QUANTITATIVE METHOD FOR EXAMINING THE URINE IN RENAL DISORDERS

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The diagnosis and treatment of Bright's disease depends, in part, on the understanding the physician has of the renal lesion with which he is dealing. As clinical practitioner,[1] he would prefer to formulate a picture of the disease without bothering with numerical values and calculations. A knowledge of the rate at which formed elements are passed in the urine, together with the quantitative estimation of the rate of elimination of protein, greatly increases the value of the urinalysis. The Addis method[2] of collection of urine and enumeration of the formed elements of the urine and estimation of urine protein provides the necessary information, but is of little value to the clinical practitioner, because it is time-consuming and does not provide constantly uniform conditions which enable him to tell at a glance the nature of the renal lesion. Constantly uniform conditions can be obtained in the modified method described here by a manipulation of the urine so as to eliminate variable times and rates of excretion. This method reduces the sediments of all patients to a uniformity which permits direct comparison and provides a series of pictures representing at a glance the nature and intensity of the renal lesion. The method allows rapid calculation of the rate at which cells and casts are excreted in the urine and at the same time assures a concentrated specimen of urine. The urine must be concentrated and the reaction must be acid, otherwise some of the formed elements will dissolve.

METHOD

Enumeration of Formed Elements.—The patient is instructed to go without fluids after breakfast the day before the test. In the evening of the day, on retiring, he empties the bladder, discards the urine and notes the time of doing so. The next morning he passes urine into a collecting bottle, again notes the time and brings the specimen to the laboratory for examination.

The volume of the collected urine is measured in a 1,000 cc. graduate cylinder. The urine is well mixed. A volume equal to the number of hundred cubic centimeters in the total urine (i. e., 1 per cent of the total) is placed in a 15 cc.

From the Department of Medicine, Stanford University School of Medicine.

1. Addis, T.: Science and Practice in Bright's Disease, Ann. Int. Med. **6:** 1077 (Feb.) 1933.

2. Addis, T.: A Clinical Classification of Bright's Disease, J. A. M. A. **85:** 163 (July 18) 1925.

graduate centrifuge tube. The urine is then centrifugated until all the elements are deposited at the bottom of the tube. The greater part of the supernatant urine is now pipetted off with a capillary pipet until it is reduced to the proper volume for calculation. If the centrifugated portion is from an eleven or twelve hour specimen, the volume must be 0.5 cc.; if from a nine to eleven hour specimen, the volume must be 0.4 cc., and if from a seven to nine hour specimen, 0.33 cc. The sediment is then mixed with the aid of a pipet, and a drop is transferred to a ruled blood counting chamber. The average number of elements in 0.1 c. mm. (one small square) is then counted. This number is divided by 2. The resulting figure is the number of million elements excreted by the patient in twelve hours. In making the count the low power objective should be used for counting casts, and the high power for the cells. This method is applicable to all specimens of urine, and gives the same picture for all similar renal lesions.

The number of elements in a twelve hour specimen is calculated by the Addis method as follows:

$$\frac{\text{Number of elements in 1 small square} \times \text{volume of specimen}}{0.0001 \text{ cc.}} \times \frac{\text{12 hour volume}}{\text{Volume centrifugated}}$$

= number of elements excreted in twelve hours.

In these fractions it is possible to have all except two items constant. The two variable items are: (1) the number of elements counted in one small square, and (2) the twelve hour volume. It is possible to make the quotient of the fraction, $\frac{\text{Twelve hour volume}}{\text{volume centrifugated}}$, constant by having the denominator (volume centrifugated) always equal to 1 per cent of the numerator (twelve hour volume). When this is done the only variable item influencing the result is the number of elements counted in one small square. Furthermore, when, instead of a twelve hour specimen the patient brings an eight, nine, ten or eleven hour specimen, it is necessary to correct the volume to twelve hours. But the calculation for this can be avoided by taking the centrifugated sediment up in, say, eight-twelfths of 0.5 cc., or 0.33 cc., for an eight hour specimen; nine-twelfths, or 0.37 cc., for a nine hour specimen; ten-twelfths, or 0.41 cc., for a ten hour specimen, or eleven-twelfths, or 0.45 cc., for an eleven hour specimen. Because the error in the final result is small, it is unnecessary to correct closer than a two hour difference. Thus, a seven to nine hour volume is taken up in 0.33 cc., and a nine to eleven hour volume is taken up in 0.4 cc., and the different items can be so arranged that the number of elements counted when divided by 2 will give the result in millions of elements per twelve hour specimen.

For example: 1. Given a twelve hour specimen of urine, the volume is 400 cc. and 12 red blood cells are counted in one of the nine squares of the counting chamber. Therefore, according to the formula: $\frac{12 \times 0.5 \text{ cc.} \times 400 \text{ cc.}}{0.0001 \text{ cc.} \times 4 \text{ cc.}} = 6{,}000{,}000$ red blood cells.

2. Given a ten hour specimen, the volume is 333 cc., with the precipitate taken up in 0.4 cc. (note that the volume does not change in the calculation) and 12 red blood cells are counted in one of the nine squares.

Therefore: $\frac{12 \times 0.5 \times 333 \text{ cc.}}{0.0001 \times 3.3} = 6{,}000{,}000$ red blood cells.

Estimation of Urine Protein.—In order to know the rate of output of albumin, it is necessary to know the quantity excreted over a definite period of time. Addis [2] calculated the excretion of protein of normal and diseased kidneys for twelve hour periods. By simple mathematics, the volume of urine excreted during twelve hours can be estimated from the volumes collected in from eight to eleven

hours. Then, with 1 cc. of urine, a calculation of the percentage of protein present is made, and from this information the excretion of protein in twelve hours is determined. For this estimation the simple and rapid procedure for measuring albumin described by Lashmet and Newburgh [3] has been adapted. The turbidity, or cloud, produced by adding sulphosalicylic acid to the urine containing protein is compared with that of a standard turbidity solution representing a known protein content.

Stock Solution: To about 200 cc. of distilled water in a 500 cc. volumetric flask, 50 cc. of a tenth-normal solution of sodium hydroxide, U. S. P., and 8 Gm. of cupric sulphate, U. S. P. (hydrous), are added. The volume of 500 cc. is made up with distilled water.

Standard Solution: After shaking the stock solution vigorously to insure uniform suspension, exactly 4 cc. of this mixture is transferred to a 100 cc. graduate cylinder and tap water is added up to the 50 cc. mark. The turbidity of this mixture is the same as that produced by a 0.1 per cent solution of protein, 0.1 Gm. per hundred cubic centimeters, when sulphosalicylic acid is used. This solution can be kept in a flask for use when wanted. The turbidity does not change on standing. It must, however, be shaken several times before using.

In order to determine the amount of protein in the urine, 1 cc. of the centrifugated urine is placed in a 100 cc. graduate cylinder, and a 2 per cent solution of sulphosalicylic acid is added to bring the volume up to 25 cc. The standard solution is placed in a second 100 cc. cylinder for comparison. If the turbidity of the mixture is the same as that of the standard solution the urine contains 0.1 per cent protein. If the turbidity is greater than that of the standard solution, the mixture should be diluted with tap water until it compares with the standard. Correction for the dilution is done by multiplying 0.1 per cent by the number of times the 25 cc. volume has been diluted; then the corrected figure is multiplied by the number of cubic centimeters of urine excreted in twelve hours. The result is the number of grams of protein excreted in twelve hours. If the turbidity of the mixture is less than that of the standard solution, urine should be added until the same turbidity as that of the standard solution is obtained. The twelve hour volume is then divided by the number of cubic centimeters of urine added and multiplied by 0.1 per cent. Not more than 5 cc. of urine should be added to the reagent; if no cloud is formed with this amount, the albumin content of the urine is negligible. This is a rough estimate of the rate of excretion of protein, and is applicable for practical use in determining the type or the progress of a renal lesion.

COMMENT

The table gives the average number of elements in the urine and the average output of albumin per twelve hours in normal and pathologic states. There is a wide range, particularly in the output of protein from which these averages are determined, so that individual variations may be considerable. Furthermore, there are gradations between the various stages of renal lesions.

In glomerular nephritis in the initial stage about 2 casts per field are seen under low power magnification, and a large number of red

3. Lashmet, F. H., and Newburgh, L. H.: An Improved Concentration Test of Renal Function: II. A Simple Method for Measuring Proteinuria, J. A. M. A. **100**:1328 (April 29) 1933.

blood cells and white blood cells per field are seen under high power magnification; in the degenerative (active) stage 4 or more casts per field are seen under low power, and a moderate number of red blood cells and white blood cells under high power, magnification; in the latent stage a few casts, about 2 in the nine squares, are seen under low power, and a few red blood cells and white blood cells per field under high power, magnification; in the terminal stage about 1 broad cast per field is seen under low power, and a moderate number of red blood cells and white blood cells under high power, magnification. In degenerative Bright's disease about 1 cast per field is seen under low power, and no, or very occasional, red blood cells and a moderate number of white and epithelial cells under high power, magnification. In arteriosclerotic Bright's disease few casts, 1 or 2 per nine squares, are seen under low power, and an occasional red and white blood cell under high power, magnification.

*Average Cast and Cell Counts**

	No. of Cases	Casts	Red Blood Cells	White Blood Cells	Protein, Mg.
Glomerular nephritis					
Initial	50	690,000	405,000,000	48,000,000	550
Latent	100	48,000	16,000,000	2,400,000	369
Degenerative or active	65	1,850,000	34,000,000	14,000,000	5,730
Terminal	50	398,000	26,400,000	10,000,000	3,550
Degenerative Bright's disease†	10	438,270	127,600	13,966,000	4,305
Arteriosclerotic Bright's disease	12	40,000	200,000	1,000,000	200
Normal	75	5,000	1,000,000	1,000,000	Negligible (30)

* From unpublished observations of T. Addis.
† These average figures are from cases of true lipoid nephrosis and do not include other forms of degenerative Bright's disease.

A comparison of the results of the two methods of enumerating the elements in the urine proves the new method to be satisfactory. While the difference in the two counts may be considerable, it is never so great that it creates a false impression. In the count of casts, for example, a difference between 40,000 and 100,000 or 1,000,000 and 5,000,000 can occur. This is of no consequence, however, when the diagnosis depends on whether or not there are 40,000 or from 1,000,000 to 4,000,000 casts. When no casts are seen, or when 1 cast is seen in nine small squares, one might expect the output to be a normal number of casts, or as many as 55,000. At first thought this seems a source of gross misinterpretation, but unless the other elements are present in abnormal numbers, one can hardly regard the specimen of urine as anything but normal. Even in mild arteriosclerotic Bright's disease there would be a slight increase in the number of cells and in the output of protein to aid in the diagnosis of the lesion.

The percentage of error in counting red blood cells and white blood cells, in comparison with the Addis method, may sometimes be as high

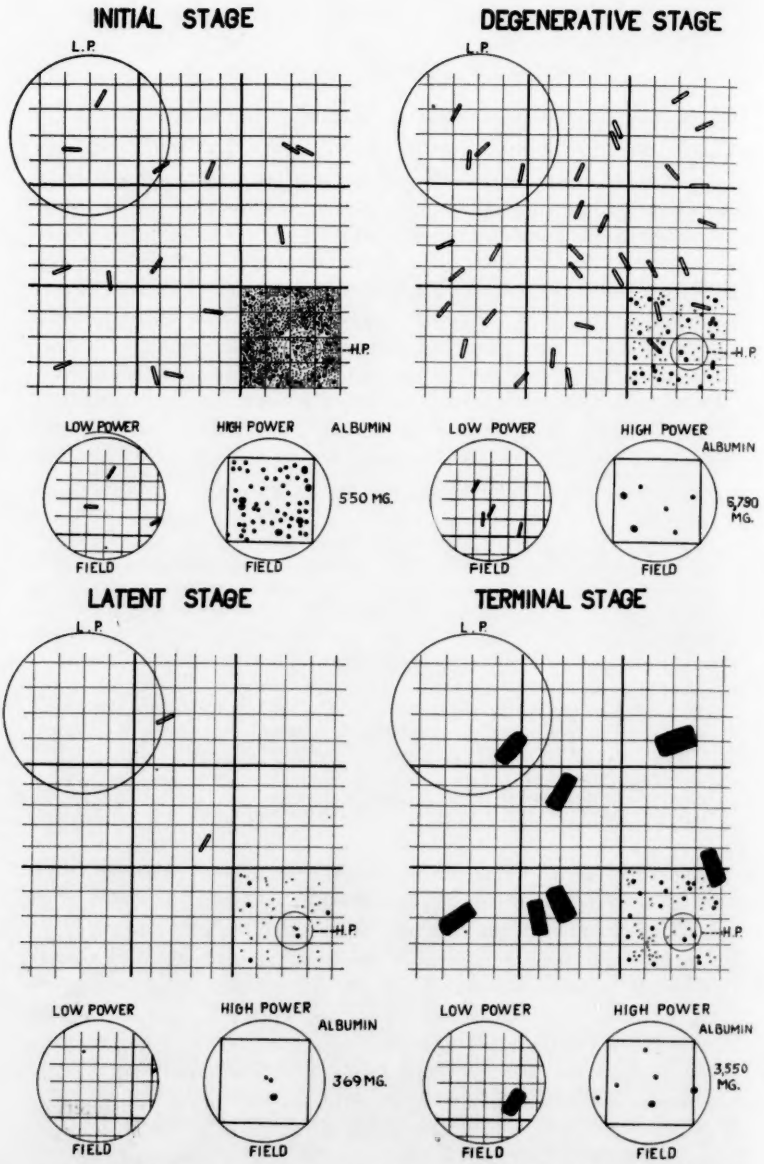

Chart 1.—Diagrams of the microscopic appearance of the urinary sediment in glomerular nephritis. Each large ruled square represents the blood counting chamber. The open oblong figures represent casts seen with the low power objective and the solid oblong figures, casts appearing with renal failure. Red and white blood cells are illustrated in the small square in the lower right of each diagram, the white cells indicated by the solid circle and the red cells by the open circle. The areas seen by low and high power objectives are indicated by circles. Both high and low power fields are illustrated below each square. The cells are not shown in the low power fields. The average twelve hour output of albumin is indicated for each condition.

as 500 with numbers below 1,000,000. This makes no difference in the picture of the renal lesion when any number up to 1,000,000 in twelve hours is considered normal; for example, by the Addis method the red blood cell count may be 100,000 and by the modified method, 500,000. For numbers above 1,000,000, errors of 300 per cent can occur in the count but do not alter the picture; for example, by the Addis method the red blood cell count may be 10,000,000 and by the modified method, 30,000,000.

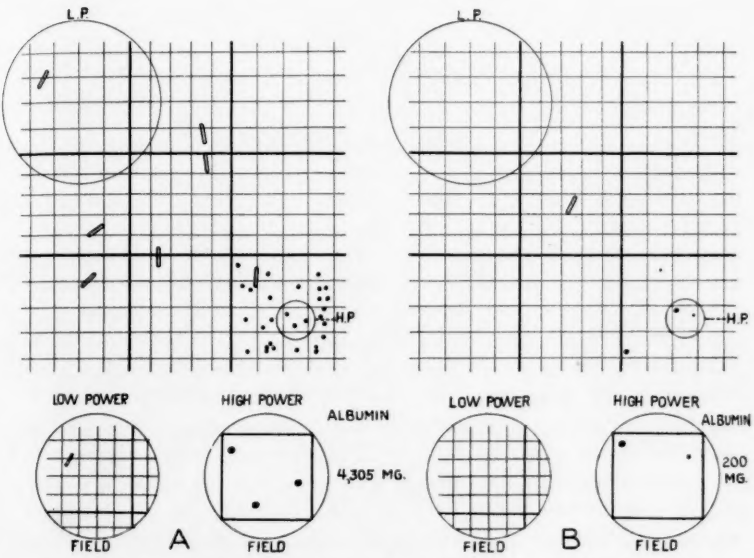

Chart 2.—Diagrams of the microscopic appearance of the urinary sediment in (*A*) degenerative Bright's disease and (*B*) arteriosclerotic Bright's disease. Interpretation of the diagram is given in the legend for chart 1. The average twelve hour output of albumin is indicated for each condition.

The error in the determination of albumin is never great enough to change the impression of the degree of damage to the kidney, provided the urine is concentrated and the volume fairly accurately measured.

In conclusion, it can be said that the clinical practitioner can obtain a constantly uniform picture, in conjunction with other clinical evidence, of every pathologic state of the kidneys by quick and easy means and with average office facilities. A little practice with the technic of counting the sediment is all that is required. After a few practice determinations a quick estimation of the type and activity of the renal lesion should be possible.

NITROGEN AND SULPHUR METABOLISM IN BRIGHT'S DISEASE

V. METABOLIC STUDY OF A PATIENT WITH EDEMA OF UNKNOWN ORIGIN

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AND

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Patients suffering from chronic nephritis with renal edema tend to retain both nitrogen and sulphur when fed diets high in protein,[1] and even retain crystalline urea.[2] On the other hand, it has been shown that patients with Bright's disease without renal edema tend toward a negative sulphur and nitrogen balance as the disease progresses.[3] The mechanism of these reactions is not clear, as it has been impossible to demonstrate an elevation of the nonprotein nitrogen level of the blood in patients retaining large quantities, whereas in patients without renal edema the nonprotein nitrogen level of the blood rises despite the negative balance. In the study of patients with renal edema it can be shown by a simple calculation that if the retained nitrogen and sulphur or urea is equally distributed throughout the body, easily measurable quantities should be demonstrable in the blood. It has always been assumed that the nitrogen and sulphur so retained are used to make up the depletion of the serum proteins, but for long periods nitrogen may be retained without an apparent variation in the serum protein values. On the other hand, it might be that such retained nitrogen and sulphur go into the edema fluid and are not recoverable. Such analyses as are available do not indicate that this is the case.[4] There remains another hypothesis, and that is that such nitrogen and sulphur are retained within all or some of the cells of the body and are stored there. The various hypotheses have been summarized recently by Moschcowitz.[5]

The present report deals with the study of a patient with a curious chronic edema, obviously not of cardiac or of renal origin. The study

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From the medical clinic of the Peter Bent Brigham Hospital.

1. Grabfield, G. P.: J. Clin. Investigation **9**:311, 1930.

2. Grabfield, G. P.: Nitrogen and Sulphur Metabolism in Bright's Disease: IV. Retention of Urea in the Nephrosis Syndrome, Arch. Int. Med. **52**:632 (Oct.) 1933.

3. Grabfield, G. P.: J. Clin. Investigation **10**:309, 1931.

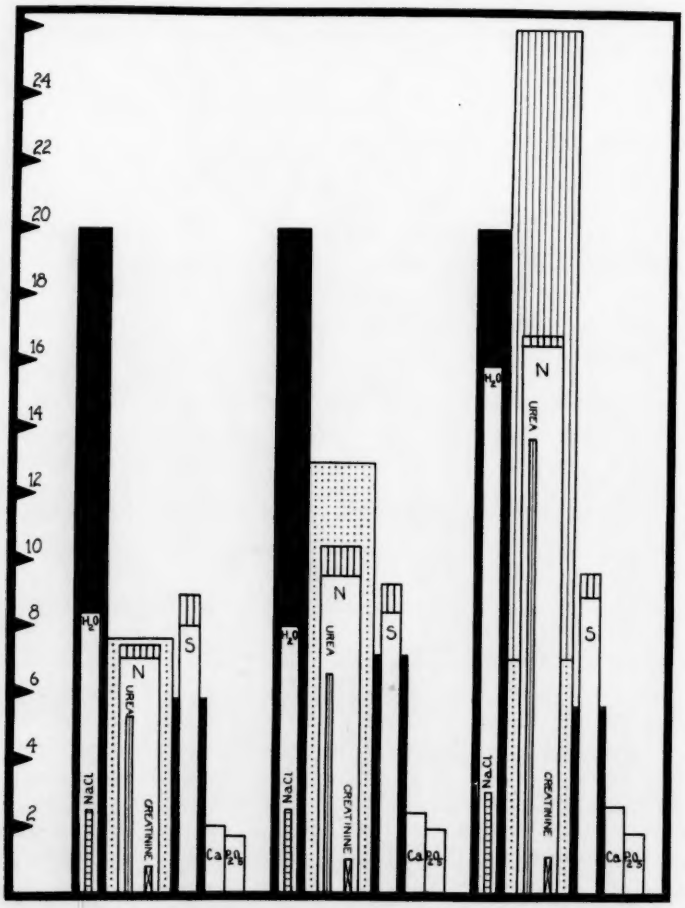
4. Peters, J. P., and Van Slyke, D. D.: Quantitative Clinical Chemistry, Baltimore, Williams & Wilkins Company, 1931, vol. 1.

5. Moschcowitz, E.: Hypoproteinemia, J. A. M. A. **100**:1086 (April 8) 1933.

Laboratory Observations for Metabolic Study During Three Periods

Date, 1933	Urine: Volume, Cc.	Urine: Creatinine, Mg.	Urine: Total Nitrogen, Gm.	Urine: Total Sulphur, Mg.	Urine: Urea + Ammonia Nitrogen, Gm.	Urine: Chlorides, Gm.	Urine: Calcium, Mg.	Urine: Phosphate, Gm.	Food: Protein, Gm.	Food: Phosphorus, Gm.	Food: Sulphur, Mg.	Food: Calories	Blood and Fluid from Chest: Total Protein, Gm. per 100 Cc.	Blood and Fluid from Chest: Serum Albumin, Gm. per 100 Cc.	Blood and Fluid from Chest: Serum Globulin, Gm. per 100 Cc.	Blood and Fluid from Chest: Cholesterol, Mg. per 100 Cc.	Blood and Fluid from Chest: Nonprotein Nitrogen, Mg. per 100 Cc.	Blood and Fluid from Chest: Urea Nitrogen, Mg. per 100 Cc.	Blood and Fluid from Chest: Creatinine, Mg. per 100 Cc.	Blood and Fluid from Chest: Phosphate, Mg. per 100 Cc.	Blood and Fluid from Chest: Calcium, Mg. per 100 Cc.	Blood and Fluid from Chest: Potassium, Mg. per 100 Cc.	Comment
Period 1																							
3/28	.....	.....	...	...	....	...	...	....	..	....	...	.....	8.3	2.7	5.6	95	29	10	1.3	3.1	9.5		
3/31	.....	.....	...	...	....	...	...	....	..	....	...	.....	8.3	2.4	5.3	...	27	..	...	...	...	....	Fibrinogen, 0.6 mg. per 100 cc.
4/ 6	420	810	8.1	530	5.8	1.6	190	1.43	49	0.86	634	1,893											
4/ 7	835	810	7.2	620	5.4	2.3	250	1.92	48	0.95	635	1,815											
4/ 8	665	660	7.3	580	5.6	3.4	160	1.66	45	0.88	576	1,797											
4/ 9	910	640	6.5	690	5.1	2.6	180	1.72	45	0.84	569	1,847											
4/10	780	690	5.9	730	4.7	1.2	150	1.60	46	0.93	585	1,844	7.5	2.8	4.7	115	18	13	1.1	3.8	9.3	17.1	
4/11	.....	.....	...	...	....	...	...	....	46	0.86	587	1,815	4.1	1.9	2.2	50	17	8	1.1	1.4	6.2	11.9	Fluid from the chest
4/11	.....	.....	...	...	....	...	...	....	..	....	...	.....											
4/12	930	960	6.7	1,320	4.9	3.2	210	1.91	47	0.93	594	1,833											
4/13	1,210	1,080	7.2	1,410	5.9	3.0	290	2.07	46	0.87	558	1,820											Daily average fecal nitrogen, 0.43 Gm.; sulphur, 0.09 Gm.
Period 2																							
4/14	930	1,010	9.0	1,260	6.9	3.1	320	2.10	77	1.29	704	1,807											
4/15	935	1,040	15.1	1,150	6.5	1.9	270	1.94	90	1.24	890	1,965											
4/16	.....	.....	...	...	...	...	...	....	89	1.26	882	1,901											
4/17	.....	.....	...	...	...	...	...	....	82	1.22	820	1,684											
4/18	960	940	9.6	710	7.9	2.3	260	2.04	90	1.20	885	1,691	6.9	3.0	3.9								
4/19	670	1,010	6.5	680	5.3	2.1	200	1.93	51	0.59	459	1,149											Daily average fecal nitrogen, 0.96 Gm., sulphur, 0.09 Gm.
4/20	670	990	7.9	420	5.0	2.3	230	1.86	87	1.05	669	1,673											
4/21	650	1,050	9.1	840	8.4	3.2	200	1.76	68	1.10	761	1,556	8.0	2.6	5.4	160	28	11	1.1	4.1	10.1		
4/21	.....	.....	...	...	...	...	...	....	..	....	...	.....	4.7	1.7	3.0	55	20	11	1.1	...	...	....	Fluid from the chest
Period 3																							
4/22	.....	.....	...	...	...	...	...	....	..	....	...	.....	...	...	...	...	..	..	...	...	...	....	Urea, 10 Gm. three times a day during this period
4/23	.....	.....	...	...	...	...	...	....	45	0.91	623	1,799											
4/24	.....	.....	...	...	...	...	...	....	45	0.87	547	1,759											
4/25	1,410	1,150	13.6	720	11.0	2.7	250	1.99	38	0.75	483	1,591											
4/26	1,820	1,200	17.3	860	14.7	2.7	270	2.03	46	0.87	568	1,803											
4/27	2,025	1,110	19.9	960	17.4	3.2	310	1.89	45	0.85	552	1,662											
4/28	2,030	1,180	16.5	1,030	14.1	3.2	280	1.99	46	0.84	553	1,765											Daily average fecal nitrogen, 0.33 Gm., sulphur, 0.06 Gm.
4/29	1,100	1,060	15.9	850	12.6	3.0	210	1.76	45	0.90	587	1,721											
4/30	1,140	1,110	16.1	900	12.6	3.0	240	1.80	42	0.87	554	1,788											
5/ 2	.....	.....	...	...	....	...	...	....	..	....	...	.....	7.8	2.6	5.2	125	32	12	1.1	3.6	10.4	19.1	
5/ 2	.....	.....	...	...	....	...	...	....	..	....	...	.....	5.4	3.7	1.7	55	23	13	1.0	1.1	6.1	10.2	Fluid from the chest
5/12	.....	.....	...	...	....	...	...	....	..	....	...	.....	7.9	2.7	5.2	...	24	8					

of this patient serves to test the hypothesis that the retention of nitrogen and sulphur is a function of the edema itself and bears no relation to the renal lesion. The history of this patient will be given in detail. It is sufficient to say here that the outstanding features shown were:



Average daily excretion for three periods under the varying conditions of intake as shown in the table. The creatinine, urea, chlorides and total nitrogen are indicated in grams, the total sulphur, calcium and phosphorus pentoxide in hundred milligrams and the fluids in hundred cubic centimeters. The outer columns represent the intake, the inner columns the output.

generalized edema, including edema of the face; definite evidence of capillary damage in the periphery, and a comparatively high total protein level in the serum, with a reversal of the albumin-globulin ratio. The

detailed laboratory observations are given in the table. A case similar to the one reported here has recently been studied by Myers and Taylor,[6] but their patient showed important retention of protein on a diet high in protein and, at times, a negative balance on a diet lower in protein.

We treated this patient as we have other patients observed in this series, giving her a carefully weighed, constant diet, adequate in calories and in protein, keeping the fluid level constant by making up the fluid of the ingested food to 2,000 cc. per day with water. The detailed figures of the experiment are given in the table, and in the chart are presented the average daily figures of three periods of study, each including six or seven days. In the first period, a diet of 50 Gm. of protein was given; in the second, an attempt was made to ingest 100 Gm. of protein, and in the third, the preliminary diet was given plus 10 Gm. of crystalline urea three times a day. During each dietary period blood and fluid from the chest were simultaneously withdrawn, and the various constituents determined. There was essentially no variation as a result of the dietary changes. The relation between the chemical constitutions of the two fluids approximates that between blood and spinal fluid. It will be seen that there was diuresis in the third period as a result of the administration of urea.

The contrast between this patient and the patient with a typical nephrosis syndrome in the metabolic reactions is apparent. There was no important retention of nitrogen in the first two periods, and there was a striking negative sulphur balance. The negative sulphur balance was increased during the period of diuresis, but during the same period there was apparently some retention of nitrogen. The low concentration of sodium chloride in the extra fluid excreted during the period of ingestion of urea may be an indication that the fluid comes from within the cells. From this contrast one may conclude that the retention of nitrogen and sulphur in patients with Bright's disease with renal edema is not a result of the edema itself.

REPORT OF A CASE

CASE 19.—*History.*—C. M. D., a married white woman, aged 32, was admitted to the hospital on March 25, 1933, with a complaint of swelling of the legs and face of four months' duration. The family history was unimportant. The patient was married fourteen years before, and was separated from her husband after four years. The husband was living and well. There was one daughter, aged 12, and there had been no miscarriages. The social history was not remarkable. The patient's habits were regular.

The patient was born in Boston, and had lived all her life either in Boston or in Nova Scotia. She had chickenpox, measles, mumps and pertussis during childhood and scarlet fever at the age of 15. Otherwise, she had had no diseases. Ten

6. Myers, W. K., and Taylor, F. H. L.: Hypoproteinemia Probably Due to Deficient Formation of Plasma Proteins, J. A. M. A. **101**:198 (July 15) 1933.

years previously the patient was said to have been operated on at the Victoria General Hospital, Halifax, for appendicitis, which was characterized by pain in the right lower quadrant and vomiting. There was no incision near McBurney's point, however. She remained in the hospital for three months, two operations being done, the first an exploration. The patient did not know what organs were removed, if any. The menstrual periods continued.

The patient came to Boston from Nova Scotia in October 1932. She was well until the first part of December. At this time she had a cold in the head, characterized by coughing and sneezing. Since then colds in the head had recurred at frequent intervals, and in the middle of February, following a particularly severe cold, pus drained from the left ear and continued until admission. One month following the first cold, in December 1932, the patient first noticed swelling of the eyelids and puffiness under the eyes, more marked in the morning. The swelling was practically confined to the face. Some weeks later her ankles swelled slightly. In the latter part of January and early February, the edema became quite marked. In the middle of February she was ill for three days with grip, and it was at this time that sudden severe pain developed in the left ear which was relieved by spontaneous rupture of the drum. One week before admission she was given a diet of nothing but 1 quart (236.5 cm.) of milk daily. The patient had no pains in the muscles or joints and no cramps. She had had no menstrual period since the previous November. There was no history of oliguria. She noticed a slight bloody tinge to the urine for one or two days at the time of onset of the otitis media. There had been no vomiting or headaches.

Physical Examination.—The fundi were normal. The patient could not hear a watch in the left ear and a slight creamy discharge drained from the ear. The canal contained moist material. The lower posterior quadrant of the tympanic membrane was absent, and this was the source of the pus. The heart was normal. The blood pressure was 124 systolic and 78 diastolic. There was dulness over the lower portion of both lungs, with absence of breath sounds over the dull area. The white blood cell count was 13,800, of which 64 per cent were lymphocytes and 34 per cent polymorphonuclears. The hemoglobin content was 102 per cent (Sahli), and the red blood cell count was 5,100,000. A blood smear showed unusual forms of cells and 9 per cent eosinophilia. Multiple myeloma was considered. The absence of anemia and definite young forms were against the diagnosis of leukemia. Eosinophilia and lymphocytosis suggested an infection from which the patient had recovered. The urine showed a low specific gravity, no albumin and no casts. The pelvic examination revealed nothing remarkable. The temperature was normal. The basal metabolic rate was plus 15 per cent. The excretion of phenolsulphonphthalein was 78 per cent in two hours and ten minutes. The two hour renal test (Volhard and Fahr) showed the specific gravity to vary from 1.024 to 1.028.

Course.—On March 29, there was an evening rise of temperature to 101 F., but on physical examination there was no change except for redness of the throat and large, red swollen tonsils, spotted with exudate. The urine showed no Bence-Jones protein, but at one time a cloud appeared after the heat and acetic acid-albumin test had been allowed to stand for ten minutes.

By April 6 the temperature was normal. The blood pressure remained low. On April 20 the edema was the same. Her condition remained essentially unchanged. On May 14, salyrgan was given as a diuretic. This was satisfactory at first, but later failed to produce diuresis. Theobromine produced no diuresis. On June 15, two teeth were extracted. About this time marked capillary relaxation was noted, as shown by the dark blue cyanosis that appeared in the legs when she hung them over the edge of the bed. Until July 9, digitalization was tried.

The blood pressure remained low, ranging between 88 and 110 systolic. The ordinary cutaneous tests with protein failed to produce a reaction on July 12. On two occasions 850 cc. of straw-colored fluid was aspirated from the right side of the chest. On July 15, pelvic examination showed a firm mass reaching almost to the umbilicus, but this could account for only the edema of the lower extremities. The patient's tonsils were removed on July 19. At no time was there anemia or leukocytosis above 15,000. The pelvic mass was believed to be inflammatory, and surgical exploration was probably indicated.

At the time of writing this report the patient was still in the hospital.

SUMMARY

1. A patient with nonnephritic and noncardiac edema was studied in the same manner as the patients with Bright's disease.

2. The behavior of the excretion of sulphur was the exact reverse of that found in patients with the nephrosis syndrome.

3. The nitrogen balance was evidently well maintained in this patient.

4. The excretion of sodium chloride may be taken to indicate that the diuresis produced by urea in this patient came from within the cells.

5. This report is included in the series so that these patients may be contrasted with those exhibiting the nephrosis syndrome and as a control to show that the metabolic reactions of patients with renal edema are not necessarily due to the edema per se.

ARTERIAL ELASTICITY IN MAN IN RELATION TO AGE AS EVALUATED BY THE PULSE WAVE VELOCITY METHOD

PHILLIP HALLOCK, M.D.
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The mounting incidence of deaths as a result of cardiovascular disease has stimulated investigators to inquire intensively into the nature and progress of the degenerative diseases of the heart and blood vessels, in an attempt to uncover possible etiologic factors and obtain data and methods for prediction in individual cases. A high percentage of all cardiovascular disease is associated with a hardening of the arteries or arteriosclerosis. The etiology of arteriosclerosis remains shrouded in mystery. Its recognition in the living subject has met with only a fair degree of success. By means of the electrocardiograph, disease of the coronary arteries can be detected fairly early in many cases. Changes in the arteriolar system, which plays such an important rôle in the regulation of arterial blood pressure may be detected in the retinal vessels with the ophthalmoscope. Then, by physical examination it is possible to judge in a rather rough and approximate fashion an advanced degree of sclerosis and thickening of the medium-sized vessels, such as the radial and brachial arteries. The latter method of investigation is admittedly inadequate for the finer and more exact evaluation of the condition of the arterial wall. It is for this reason, therefore, that I resorted to a method which affords quantitative data regarding arterial elasticity through physical measurements. The validity of the method, which is based on the theory of wave transmission through elastic tubular structures, has been well established by experimental procedures. The method which will be described is well applicable to a study of the rigidity of the arterial tubing in the descending aorta and in the brachioradial systems.

THEORETICAL CONSIDERATIONS OF ARTERIAL ELASTICITY

It is now recognized that the extensibility of an artery can be measured by the velocity of the pulse wave propagated through it. Moens,[1] in 1878, by experimental methods, derived the mathematical expression (1) $V = K \sqrt{\frac{t.E.a.}{D.d.}}$ for the velocity of the front of the pulse wave occurring in arteries, in which the velocity of the pulse wave (V)

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1. Moens, A. I.: Die Pulskurve, Leiden, E. J. Brill, 1878, p. 90.

varies directly as the square root of the product gravity (g), the elasticity coefficient (E) and the thickness of the arterial wall (a), and inversely as the product of the square root of the density of the blood (D) and the diameter of the lumen of the vessel at the end of diastole (d). D may be assumed to be constant and equal to 1.055; K is a constant. Moens proved the validity of his formula by experiments on elastic tubing.

It is obvious that for practical purposes Moens' expression is of little value, as the factors E, a and d are immeasurable quantities in situ. Bramwell and Hill,[2] after careful consideration of the theory of wave transmission, came to the conclusion that within limits of experimental error, Moens' formula would be applicable provided the factors could in some manner be transformed into measurable units. To accommodate factors which may be measured, they ingeniously transformed Moens' formula into the following:

(2) $V = 0.357\sqrt{\frac{v}{dv/dp}}$ or $V = 0.357\sqrt{\frac{v.\ dp}{dv}}$, in which $\frac{dv}{dp} \cdot \frac{1}{v}$ is the relative increase in volume (v) of the artery per millimeter of mercury increase of pressure (p). Converting this expression into a slightly different form (reciprocal), the ratio of pressure increase to volume increase $(\frac{dp}{dv}.v)$ has been designated as the coefficient of volume elasticity. It increases directly as the rigidity and inversely as the extensibility of the arterial wall.

Converting equation (2) into terms of percentages, it becomes

(3) $$V = \frac{3.57}{\sqrt{\text{percentage increase in volume per millimeter of mercury increase of pressure}}}$$

or (4) percentage increase in volume per millimeter of mercury rise in pressure $= \frac{12.7}{(\text{velocity in meters per second})^2}$.

From expression (3) or (4) it is obvious that the velocity of the pulse wave is a function of arterial rigidity or the coefficient of volume elasticity. To determine the arterial rigidity in situ, then, it is not necessary to know the thickness of the arterial wall, its radius or the elastic coefficient E. By simply determining the rate of transmission of the pulse wave, the volume increase per unit rise of pressure (the coefficient of extensibility of vessels) can be determined in absolute units.

FACTORS MODIFYING THE VELOCITY OF THE PULSE WAVE

The velocity with which the pulse wave is transmitted is dependent primarily on the coefficient of elasticity or rigidity of the artery. However, several other factors deserve consideration. The velocity of the blood flow may be regarded as a small quantity in calculating the

2. Bramwell, J. C., and Hill, A. V.: The Velocity of the Pulse Wave in Man, Proc. Roy. Soc., London, s.B **93**:229, 1922.

velocity of pulse waves. Yet it may affect to a noticeable degree the form of the pulse wave as well as its rate of propagation. Assuming 0.75 meter per second to be the average maximum velocity of the flow of blood in the human aorta under basal conditions and 0.25 meter per second the average velocity in the carotid artery, it is obvious that the correction for the velocity of blood flow itself is small. But Bramwell and Hill [2] pointed out that any considerable increase in the velocity of the blood as a result of local or general disturbances will cause an equal increase in the velocity of the pulse wave. They stated that "any experimentally determined wave (pulse) velocity must represent the velocity of the wave relative to the blood, plus the velocity of the blood in the artery."

Another factor that may modify the velocity of the pulse wave is the act of respiration. Hickson and McSwiney [3] showed that the velocity of the pulse wave is slightly higher during the phase of expiration, owing to the fact that during the expiratory phase the blood pressure is slightly increased.

But the chief factors concerned in altering the velocity of pulse waves are variations in the arterial elasticity. The variations are essentially the result of two factors: (1) the effective internal pressure within the artery, and (2) the constitution or "make-up" of the arterial wall.

In regard to the former, considerable experimental evidence indicates that the pressure inside the vessel plays an important rôle in modifying the arterial elasticity and thus in modifying the speed of transmission of the pulse wave. Roy,[4] in 1880, pointed out by means of a series of extensibility curves obtained by experimental methods that the elasticity of isolated human and animal aortas depends to a large extent on the intra-arterial pressure. Grunmach,[5] in 1885, measured the velocity of the pulse waves in an attempt to establish the relationship between the velocity of the pulse wave and the aortic pressure. He found that patients suffering from defects of the aortic valves and severe anemia, both of which conditions give rise to low aortic pressure, had slow velocities of the pulse waves, while those suffering from arteriosclerosis had high velocities, a fact which he attributed to increased aortic pressure.

Bramwell and Hill,[2] in 1922, after recalculating Roy's curves were able to derive from them the percentage increase in volume of the

3. Hickson, S. K., and McSwiney, B. A.: Effect of Respiratory Movements on Pulse Wave Velocity, J. Physiol. **59**:217, 1924.

4. Roy, C. S.: The Elastic Properties of the Arterial Wall, J. Physiol. **3**:125, 1880-1882.

5. Grunmach, E.: On the Pulse Velocity in Diseases of the Circulatory Apparatus, Virchows Arch. f. path. Anat. **102**:565, 1885.

vessel per millimeter of mercury increase in pressure and thus the velocity of the pulse wave at various pressures. Their results showed that the velocity of the pulse wave was low at low pressures and increased considerably at higher pressures. By an ingenious method, the same investigators experimentally confirmed these deductions elicited from Roy's curves by direct mechanical measurement of the velocity of the pulse wave in isolated arteries which were filled with mercury in order to slow the transmission of the wave. They found that at a diastolic pressure of 25 mm. of mercury the velocity of the pulse wave was 3.76 meters per second. At a diastolic pressure of 200 mm. of mercury, it increased to 18.5 meters per second. Their results indicated conclusively that the velocity of the pulse wave increased almost directly as the blood pressure, and that the rate of propagation of the pulse wave was a mechanical phenomenon depending on the elastic properties of the vessels. Thus, with increasing diastolic pressure there appeared to be a gradual diminution of extensibility and a gradual increase of rigidity.

Bramwell, McDowall and McSwiney,[6] in 1922, described a method by which the extensibility of the brachioradial artery could be measured at all internal pressures from zero up to the diastolic pressure in living man. By the employment of a compression bandage they were able to alter at will the effective pressure inside a certain region of an artery and to calculate the velocity of the pulse wave in the segment of artery submitted to the various pressures. Making use of the formula:

$$V = \frac{d}{y - x \quad \frac{(l - d)}{l}}$$

in which x equals the transmission time between two points at a distance (l) from each other and y the time between the same points when a bandage of length d is applied, the velocities of the pulse wave at various diastolic pressures were calculated. They found that the velocity of the pulse wave increased and therefore extensibility decreased as the intra-arterial pressure was increased.

Bramwell, Downing and Hill,[7] in 1923, by the use of the two hot-wire sphygmographs and two strings of an Einthoven galvanometer, repeated their study on isolated arteries in order to arrive at more accurate measurements. This study was limited to the carotid artery, a vessel which contains only a relatively small amount of muscular tissue. In twelve observations, the velocities were recorded at twelve different pressures ranging from 15 to 250 mm. of mercury. Convert-

6. Bramwell, J. C.; McDowall, R. J. S., and McSwiney, B. A.: The Variation of the Arterial Elasticity with Blood Pressure in Man, Proc. Roy. Soc., London, s.B **94**:450, 1923.

7. Bramwell, J. C.; Downing, A. C., and Hill, A. V.: The Effect of Blood Pressure on the Extensibility of the Human Artery, Heart **10**:289, 1923.

ing formula (3) into another convenient form, the velocity of the pulse wave may be expressed in terms of millimeters of mercury rise of pressure per 1 per cent increase of volume or rigidity of the artery as follows:

(5) $V = 3.57 \sqrt{\text{millimeters of mercury of pressure required to produce 1 per cent increase in volume.}}$

After calculating the rigidity of the vessel from the experimentally determined velocity of the pulse wave by means of this formula, they plotted the various blood pressures against rigidity, and were able to demonstrate a striking increase of rigidity of a vessel with the increase of blood pressure. The evidence proves that the arterial elasticity depends to a considerable extent on the diastolic blood pressure.

The second important factor modifying the rate of transmission of the pulse wave is concerned with changes inherent in the arterial wall. According to the classic formula of Moens,[1] the velocity of the pulse wave varies directly as the square root of the thickness of the arterial wall and inversely as the square root of its diameter. Consequently, any factor which causes thickening of the wall of the artery or narrowing of its lumen will increase the rate of transmission of the pulse wave.

Bazett and Dryer,[8] in 1922, showed that the velocity of transmission is considerably lower in the large vessels than in the smaller peripheral ones. This observation has been confirmed in my studies on the transmission time of the pulse wave through the aorta and brachioradial vessels. The fact that medium-sized arteries, such as the radial, the brachial or the carotid vessels, are endowed with relatively more muscular tissue than the aorta and the iliac vessels, and the fact that the internal diameter of the peripheral arteries is considerably less than that of the aorta and its immediate branches probably explain the difference in the rates of transmission of the pulse wave in these vessels. Furthermore, because of these anatomic variations, it is reasonable to assume that the medium-sized arteries, as opposed to the large vessels, are subjected to greater vasomotor influences, which may functionally or otherwise so alter the tonus and degree of contraction of their walls as to increase the volume elastic coefficient (rigidity) and consequently the speed of the pulse wave.

Laubry, Mougeot and Giroux,[9] in 1921, by increasing the tonicity of the artery in situ by cold applications, showed that the velocity of the pulse wave increased. On the contrary, vasodilatation diminished the speed of the pulse wave.

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8. Bazett, R. C., and Dryer, N. B.: Measurement of Pulse Wave Velocity, Am. J. Physiol. **63**:94, 1922.

9. Laubry, C. H.; Mougeot, A., and Giroux, R.: The Speed of the Arterial Pulse Wave, Arch. d. mal. du cœur **14**:49, 1921.

MacWilliam,[10] in 1902, showed that medium-sized vessels such as the carotid, obtained soon after the death of the animal, could be submitted to various states of relaxation and contraction by various physical and chemical means. By an ingenious method he measured the increase in volume of short segments of the altered vessels under various degrees of pressure, and then plotted volume-elasticity curves in which the volume was plotted along the ordinate axis and the pressure along the abscissa. The curve of the strongly contracted vessel was concave to the abscissa, while that of the relaxed vessel was convex to the abscissa. He concluded that the differences in the shape of the curves were due to the fact that the muscular resistance of the contracted vessels opposed extensibility at normal internal pressures, while in the relaxed vessels the muscular contraction was lacking to a considerable extent and thereby favored extensibility at the same normal internal pressures.

MacCordick [11] found that the carotid arteries of the horse were contractile several days following removal and that the various degrees of contraction made a decided difference in the measurements of elasticity. Renterwall [12] found that when extension curves were plotted by testing the same artery in rapid succession, a difference was found between the successive curves. By treating the same arteries with epinephrine he was able to modify the shape of the curves. He concluded that the differences in the curves were due to the varying degrees of contraction. These experiments show that the transmission of the pulse wave in medium-sized vessels is affected to a considerable extent by the degree of muscular contraction as well as by the intra-arterial pressure. Friberger,[13] in 1912, studied the velocity of the pulse waves in arteries with palpable thickening of the walls (in patients suffering from nephritis) and attributed the increase of the velocity of the pulse wave to the increased muscular tension combined with degenerative changes in the arterial wall. Sands,[14] in 1925, was unable to confirm this observation.

The constitution of the wall of the aorta is such that its physical behavior is different from that of the medium-sized vessels. While there is a slight amount of smooth muscle in the aortic wall, this has a negligible effect as far as the transmission of the pulse wave is con-

10. MacWilliam, J. A.: On the Properties of the Arterial and Venous Walls, Proc. Roy. Soc., London **70**:109, 1902.

11. MacCordick, A. H.: Ueber die Stromung des Blutes in der Gefassbahn und die Spannung der Gefasswand, Beitr. z. path. Anat. u. z. allg. Path. **66**:377, 1920.

12. Renterwall, quoted by Thoma, R.: Ueber die Elastizität der Arterien und die Angiomalacia, Virchows Arch. f. path. Anat. **236**:242, 1922.

13. Friberger, R.: Ueber die Pulswellengeschwindigkeit bei Arterien mit fühlbarer Wandverdickung, Deutsches Arch. f. klin. Med. **97**:280, 1912.

14. Sands, J.: Studies in Pulse Velocity: Pulse Wave Velocity in Pathological Conditions, Am. J. Physiol. **71**:519, 1925.

cerned. Thus, Zon,[15] in 1932, experimentally, in rapid succession, obtained extension curves of a strip of ascending aorta and found no difference between the respective curves. After the application of a 0.005 per cent solution of epinephrine (surrounding the aorta) he repeated the experiments and found no appreciable change in the form of the curve.

The elastic properties of the aorta are governed essentially by the diastolic pressure and by the amount of elastic tissue contained in its wall. The aorta is probably almost entirely devoid of vasomotor influences such as control the medium-sized and smaller arteries. Physical tests, to be described later, show that the extensibility of the aorta is considerably greater than that of the medium-sized vessels. This is undoubtedly due to the fact that the aorta, as indicated by the Weigert and Van Gieson stains, contains a much greater amount of elastic tissue. The fact that the lumen of the aorta is greater than that of the medium-sized vessels also explains in part its increased extensibility.

The arterial changes that occur with age have thrown considerable light on the physical properties of the aortic wall. Frank,[16] in 1927, showed that the elastic volume coefficient (rigidity) is dependent on the tension per unit cross-section area of the vessel, approximately as given by the equation $E = E_o + qt$,[2] in which E denotes the elastic coefficient, E_o the residual elasticity, q the elastic characteristic according to age and t the tension. He found that the value q changed in the individual arteries much more than E_o, and also that q increased with age. Thus, in old people he found the value of q to be six times as great as that found in children, while E_o remained approximately the same. With a tension of zero, E remained the same for both longitudinal and transverse expansion. With increasing tension the former increased more rapidly than the latter. Thus the extensibility of the aorta is much greater in the longitudinal direction than in the transverse. He concluded, therefore, that the elastic substance in the arterial wall could not be homogeneous. He stated that some colloidal alterations in the arteries are responsible for changes in the elasticity relations. He also pointed out that arterial rigidity increased very little with increased tension at lower tensions in young persons. On the other hand, in older subjects the arterial rigidity increases rapidly with an increase in tension at lower tensions, so that the curves assume almost straight lines at their very beginning.

It appears from recent work that the alterations that occur with age, especially in the aorta, involve corresponding regressive changes in the

15. Zon, L.: Unpublished material (Thesis, University of Minnesota, 1932).

16. Frank, O.: Das Altern der Arterien, Sitzungsb. d. Gesellsch. f. Morphol. u. Physiol. **37**:23, 1927; abstr., Ber. ü. d. ges. Physiol. u. exper. Pharmakol. **41**: 234, 1927.

elastic tissue. Aschoff [17] called attention to the fact that when an aorta was loosened from its attachments from the vertebral column, except at its ends, and then cut transversely through its midportion, the cut ends would retract considerably in young people, but not to any appreciable extent in old persons. This difference he attributed to weakening of the elastic tissue. With advancing age the size of the aorta gradually increases, and physical tests indicate that extensibility decreases. Zon,[15] in an attempt to correlate the physical behavior of aortas at various ages with their respective histologic structures, determined the extensibility of rings of aortas from all periods of life. His studies show a progressive disappearance of elastic tissue, with a slight replacement by collagenous tissue and a change in form of the elastic fibers with age. He expressed the belief that the decrease of extensibility with age is due to the degeneration of elastic tissue.

It is obvious from the preceding considerations that the factors which govern the transmission of the pulse waves in medium-sized vessels are not altogether similar to those that govern the transmission of pulse waves in aortas.

A number of investigators have studied the rate of transmission of the pulse wave in man, especially in the medium-sized vessels such as the radial, brachial and carotid arteries. Several have even attempted to study the rate of transmission through the aorta and the iliac vessels. These investigations have afforded valuable information concerning the elasticity of arteries. However, although the results indicate that there is a progressive increase of velocity with age, an inspection of the values for the velocity of the pulse wave, as reported, reveals considerable discrepancy as to what constitutes the norms of velocity for different age groups. Furthermore, the increments of the velocity of the pulse wave reported in successive age groups are of such variable magnitude as to preclude the possibility of establishing a normal standard. A glance at Wiggers' [18] table (1923), derived from the graphic records of normal adults, as given by various investigators, shows the variability of the values reported.

Obviously such conflicting results are of little value in attempting to establish a normal range of velocities of the pulse wave. The disparity shown in the results can be explained only in part by the difference in methods and technics employed in registering the rate of transmission of the pulse wave. However, since the introduction of the segment capsule by Frank,[19] in 1913, and the hot wire sphygmograph by Hill,[20]

17. Aschoff, L.: Lectures on Pathology, New York, Paul B. Hoeber, Inc., 1924, p. 131.

18. Wiggers, C. J.: Modern Aspects of Circulation in Health and Disease, ed. 2, Philadelphia, Lea & Febiger, 1923.

19. Frank, O.: Theory of Segment Capsule, Ztschr. f. Biol. **59**:526, 1913.

20. Hill, A. V.: Hot Wire Sphygmograph, J. Physiol. **54**:Cxvii, 1920-1921.

in 1920, accurate optical records of the pulse wave can be obtained, thus minimizing errors that otherwise would be present. Another source of error that may account for the inconstancy of results is the variability in the methods of measuring the anatomic landmarks which represent the actual arterial distance traversed by the pulse wave in passing from the heart to points at which the pulse wave excursions are recorded. Moreover, in older persons the tortuosity of the vessels introduces an immeasurable though probably not great error.

Recently, Hemingway, McSwiney and Allison,[21] employing the hot wire sphygmograph, showed by accurate measurements that the velocities of the pulse wave and the extensibilities of the brachial and radial arteries varied considerably in normal persons, thus further complicating the problem of arterial elasticity. They found, after the construction of a series of curves relating the velocity of the pulse wave and extensibility to effective arterial pressures, that the arteries of normal subjects could be classified in three types: (1) normal, (2) hyperextensible and (3) hypo-extensible. They stated that while Bramwell, Hill and McSwiney [22] found the coefficient of the mean extensibility of an artery

TABLE 1.—*Values for the Velocity of the Pulse Wave Reported in the Literature* *

Year	Investigator	Carotid Radial Velocity, Min. and Sec.	Aortic Velocity, Min. and Sec.
1864	Caermak	6.7	
1878	Moens [1]	8.7	
1879	Grunmach [5]	8.29	
1887	Keyt	7.37	
1889	Edgren	7.63 to 7.32	
1889	Horweg	9.0	
1912	Munzer	9.0 to 12	
1912	Friberger [13]	6.7 to 9.4 (men) 7.0 to 10.0 (women) (16 to 36 years)	
1912	Ruschke	9.0	
1921	Laubry, Mougeot and Giroux [9]	8.0	
1922	Bramwell and Hill [2]	5.9 to 7.5 (15 to 50 years)	
1922	Bazett and Dryer [8]	5.2 to 7.9 (13 to 36 years)	4.5 to 9.7
1924	Sands [14]	3.62 to 6.85 (16 to 41 years)	3.57 to 3.69
1927	Beyerholm	5.91 to 7.38 (1½ to 60 years and more)	
1932	Hafkesbring and Ashman	5.8 to 10.3 (17 to 44 years)	

* Table 1 is in part copied from Wigger.[13] I have added to Wigger's table the last four authors and the values for the velocities of pulse waves they obtained.

21. Hemingway, A.; McSwiney, B. A., and Allison, P. R.: Extensibility of Human Arteries, Quart. J. Med. **21**:489, 1928.

22. Bramwell, J. C.; Hill, A. V., and McSwiney, B. A.: The Velocity of the Pulse Wave in Man in Relation to Age as Measured by the Hot-Wire Sphygmograph, Heart **10**:233, 1923.

at the age of 20 to be 0.33, in the experiments of Hemingway, McSwiney and Allison, the variation at this age was between 0.3 and 0.64. Hemingway, McSwiney and Allison attributed this wide variation to influences originating presumably from the vasomotor center and affecting the tone of the smooth muscle in the arterial wall.

It is apparent from the preceding considerations that the status of data on the velocity of the pulse wave, as far as the practical application for evaluating arterial elasticity is concerned, is attended by considerable disagreement and confusion. It is necessary, first, to establish criteria for normal standards of the velocity of the pulse wave at various ages. Once these are established it will be possible to evaluate the elasticity of the vessels in which arterial disease is suspected. Such an object can be accomplished only by the use of a precise and accurate recording instrument with which the velocity of the pulse wave of a large series of patients of all ages can be measured. After the data are obtained it is necessary to determine the normal values for each age group. If the observers mentioned previously are correct in stating that the elasticity of the vessels of normal persons falls into one of three groups, then it is necessary to determine the limits of variability of arterial elasticity in each age group. If, moreover, there is a relationship between the elasticity and diastolic pressure, it is necessary to subject this relationship to a quantitative analysis by statistical means.

METHOD

Registration.—In this work, optical registration was employed throughout. An optical projection system, as described by Wiggers [18] for reflecting light from two segment capsules, was used in such a manner that the illuminating beam of light was thrown through two adjustable vertical slits onto small rectangular mirrors (1 by 4 by 0.01 mm.) mounted on the margin of segment capsules. A time marker activated by a motor synchronized by a tuning fork was interposed between the condensing lens and the adjustable slots at the focal point of the light for recording the periods of time in fifths of a second. A narrow hood 0.75 meter long was placed between the recording camera and the segment capsules, and the apparatus was carefully alined before each operation to obviate parallax. The segment capsules were connected to the receiving instruments by tubing as short as possible and of exactly the same length in the two systems, thus assuring equal transmission time of the pressure waves traveling in the receiving systems. The receiving apparatus consisted of two parts, a shallow aluminum cup being used over the carotid vessels and a glycerin pelote over the radial and femoral arteries. Segment capsules 12 mm. in diameter were employed.

Measurement of Records.—The beams of light reflected from the segment capsules were directed on the cylinder lens of a Hindle photokymograph and recorded on sensitized bromide paper, 12 cm. wide. Each record contained two sets of curves, the first recording the pulse waves from the radial and from the carotid arteries taken simultaneously, and the second recording similarly the pulse waves from points on the carotid and on the iliac arteries. The first set of curves determines the brachioradial transmission time; the second set determines the

aortic-iliac transmission time. Each curve consists of from six to eight cycles. Of these, the four or five cycles showing the most abrupt upstrokes were measured, and an average figured. Differences in measurements of the various cycles did not vary as a rule more than 3 or 4 per cent.

The averages of four or five cycles were figured to obtain a mean value. The variations in the individual readings were partially due to changes in blood pressure associated with respiratory movements. Because of the four or five successive readings on each subject, it was possible to test the reliability of the technic (table 2). The mean and total range of the deviations from the mean of the four or five readings were calculated for twenty-five subjects chosen at random from each age group, except in the age group from 5 to 9 years, in which nineteen records were chosen and in the 65 year age group, in which eighteen records were chosen.

TABLE 2.—*Deviations of the Instrumental Units*

Age Period, Years	Mean Deviation of Five Readings		Total Range of Deviation of Five Readings	
	Maximum	Mean	Maximum	Mean
	Velocity of the Radial Pulse Waves			
5 to 9	0.28	0.12	0.7	0.4
10 to 14	0.30	0.15	0.9	0.4
15 to 19	0.26	0.13	1.0	0.4
20 to 24	0.35	0.14	1.0	0.4
25 to 34	0.24	0.11	0.8	0.4
35 to 44	0.26	0.15	0.7	0.4
45 to 54	0.30	0.14	0.8	0.4
55 to 64	0.28	0.14	0.8	0.4
65 and more	0.25	0.13	0.9	0.4
Average	0.28	0.13	0.87	0.4
	Velocity of the Aortic Pulse Waves			
5 to 9	0.22	0.13	0.8	0.4
10 to 14	0.30	0.12	1.0	0.4
15 to 19	0.23	0.12	0.6	0.4
20 to 24	0.35	0.15	1.2	0.4
25 to 34	0.23	0.13	0.8	0.4
35 to 44	0.28	0.14	0.8	0.4
45 to 54	0.23	0.13	0.8	0.4
55 to 64	0.36	0.15	1.0	0.4
65 and more	0.37	0.17	0.9	0.4
Average	0.30	0.14	0.88	0.4

According to Tippett,[23] the standard deviation, σ, may be determined from the mean range for the twenty-five sets of five readings each by dividing the mean range by a constant, 2.326; thus, $\sigma = \frac{0.4}{2.326} = 0.17$, when the constant is obtained from the tables according to the number of readings in each sample in the series. Since the mean range was the same for all age periods and for both radial and aortic velocity, the standard deviation is the same, namely, 0.17. Since the mean deviations for both groups vary only from 0.11 to 0.17, the coefficients of variation, $C.V. = \frac{\sigma}{\text{mean}} \times 100$, for all age periods and for velocities of both aortic and radial pulse waves are between 1 and 1.5 per cent. Hence, by definition of the standard deviation and the coefficient of variation, two thirds of the time the error of the instrument would be within a range of from ± 1 to ± 1.5 per cent ($\pm$ 1 C.V.) and 95 per cent of the time within a range from 2 to 3 per

23. Tippett, L. H. C.: Method of Statistics, London, William Norgate, Ltd., 1931, p. 26.

cent. Hence, the probable range of error introduced into the readings of the velocity of aortic and radial pulse waves is about 2.5 per cent.

In some of the records the exact beginning of the systolic upstroke was complicated by the presence of a presphygmic vibration (period of rising tension of the ventricular systole). In these cases the records were rejected because of the difficulty in determining the exact beginning of the upstroke.

The records were measured by means of a glass reading plate having one face accurately etched in millimeters. The etched face was laid down directly on the record so as to avoid parallax. Both the reading plate and the record were placed on a stage to which was attached an adjustable microscopic lens capable of magnifying the record six times. In this manner the distance could be measured to within 0.1 mm. With the sensitized paper traveling at the rate of 100 mm. per second, the intervals of time can be read to within one thousandth of a second. In many of the records, the commencement of the upstroke was marked with a fine-pointed needle held under a microscopic lens, thus insuring accuracy.

All records were recorded with the patient lying at ease in the supine position. In measuring the brachioradial velocity an aluminum cup was held snugly over the bifurcation of the right carotid artery, while the glycerin pelotte was fixed firmly over the radial vessel of the right wrist by a strap encircling the wrist. In measuring the aortic time of transmission, the aluminum cup was held firmly on the left carotid bifurcation, and the glycerin pelotte was held firmly over the iliac artery just below the inguinal ligament. When the patient was excited and the pulse rapid, he was given a five minute period of rest before the receiving apparatus was applied. The blood pressure was measured by the auscultatory method after taking the record. In most cases, the height and weight were also recorded.

Anatomic Landmarks and Measurements.—As mentioned previously, the anatomic measurements may introduce a considerable error if not taken with precision. Thus, assuming the total distance measured to be about 50 cm., a difference of 2 cm. in measurement would introduce an error of 4 per cent. If the pulse wave traverses an actual distance of 50 cm. in six-hundredths of a second, the velocity would be 8.33 meters per second, while if the measured distance was 48 cm., the velocity would be determined as 8 meters per second.

All patients were made to assume as nearly as possible the same position, so that the anatomic landmarks could easily be recognized and accurate measurements taken therefrom. The landmarks of the bifurcation of the common carotid artery can be found by following the superior border of the lamina of the thyroid cartilage out to its very end. At this point the bifurcation can most easily be palpated. Because of the anatomic relationships in the neck, it is important that the head be only slightly extended. Excessive extension may introduce an error in measurement.

The measurements of transmission time for the carotid and radial vessels were all made on the right side of the body. Accurate measurement from the origin of the right subclavian artery to the radial artery at the styloid process can be made by extending the right arm at right angles to the long axis of the body. The length of the subclavian brachioradial branch can be determined accurately by measuring the direct distance from the sternoclavicular joint to the styloid process of the radius. Likewise, if the head is not thrown back too far, the carotid vessel can be mapped out with precision from the same point over the sternoclavicular joint directly to its bifurcation. Similarly, the length of the left common carotid artery can be measured.

To obtain the transmission time of the pulse wave through the aorta, it was necessary to make a correction for the aortic arch. The origin of the left common carotid artery as topographic anatomic sections show, and as I have observed in a large number of postmortem examinations, is at the level of the inferior border of the first left rib just lateral to the midsternal line. The lower border of the first left rib at its junction with the sternum was therefore used as a landmark. The level of the crests of the ilium at the midline of the body was taken as the landmark for the bifurcation of the aorta. The direct distance from the lower border of the first rib to the midpoint of a line passing over the iliac crests was measured. To this distance was added the length of the iliac artery. Its length was determined by measuring the direct distance from the midline of the abdomen at the level of the iliac crests to the inguinal ligament at which point the glycerin pelotte lay over the iliac artery just at its entrance into the femoral canal.

To make this correction for the arch, 4 cm. was added to the measurements in persons over 15 years of age, and 2 cm. was added to those of subjects under 15 years. These correction values were obtained from the study of eighty-three postmortem examinations of persons ranging from 3 to 99 years of age. The external measurement of the length of the aorta was first made, as already described. After opening the thorax and abdomen, the aorta was carefully cleaned, and the distance from the origin of the left common carotid artery to the bifurcation of the aorta was measured with a piece of string laid carefully along the intima of the aorta. In forty-seven cases both the greater and the lesser curvatures of the aorta were measured, and the mean value obtained. In the remaining cases, only the greater curvature was measured. In these, when the greater curvature was measured and compared to the externally measured aortic distance, the average difference was 5.77 cm. In the forty-seven cases in which both the greater and the lesser curvature were measured and the mean computed, the average difference between the internally and the externally measured distance was 4.87 cm. The average correction factor in the patients under 15 years of age was calculated to be 1.8 cm. For convenience, the factor of 2 cm. was used as the correction factor in patients under 15 years. Any error due to the use of this factor will be less than ± 4 per cent. While the correction factor actually determined for use above the age of 15 was 4.87 cm., I have added only 4 cm., as the greater portion of the postmortem cases in which the correction factor was determined fell in the older age group, and a considerable number of these patients had died of cardiovascular and renal diseases. Any error caused by using this factor of 4 cm. will be less than ± 4 per cent in 90 per cent of all cases. In 10 per cent of the cases the error is greater than 4 per cent and may occasionally be as high as 10 per cent. In most of these cases there is considerable lengthening of the arch associated with atherosclerosis, and the external measurement cannot be expected to be as accurate as when there is no aortic lengthening. A marked deformity of the spine can also cause considerable error in this measurement.

Aortic Velocity of Transmission.—This was obtained in the following manner: First, simultaneous curves were recorded from the pulses at the bifurcation of the left common carotid artery and at a point over the iliac artery at the inguinal ligament.

Second, measurements were taken of the distance from the lower border of the left first rib to landmarks on the neck and inguinal ligament (with the correction factor added to the latter) and the former measurements subtracted from the latter to obtain the distance traversed by the pulse wave in the recorded time interval. Finally, to arrive at the velocity of transmission of the pulse wave, the

distance traversed was divided by the time interval as recorded between the commencement of the carotid and that of the iliac systolic upstroke (fig. 1, aortic curves).

Carotid-Radial Velocity of Transmission.—In a similar manner, this was calculated (fig. 1, radial curves), except that the right sternoclavicular joint was used as one of the landmarks instead of the lower level of the first left rib, and a point near the styloid process of the radius where the pelotte was fixed as the landmark instead of the midpoint on the inguinal ligament.

The transmission time of the pulse waves through the aorta and iliac artery may be utilized as an accurate factor in determining the minute output of the heart from the Broemser and Ranke formula,[24] as the accuracy of their equation depends to a large extent on knowing the distensibility of the central vessels and the aorta.

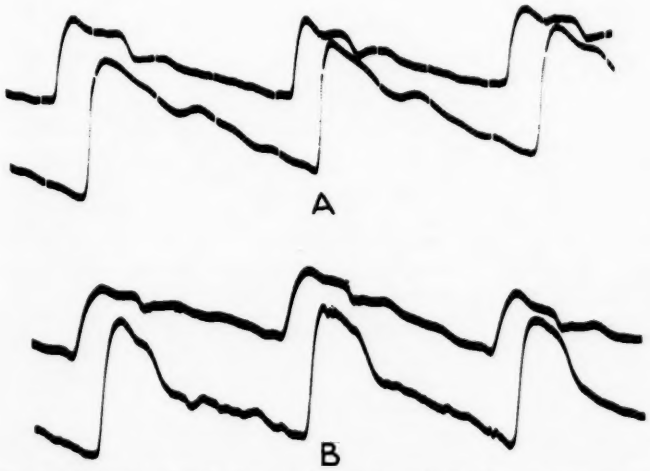

Fig. 1.—Examples of optically recorded tracings obtained by the segment capsule method as described in the text. In the lower record a finer time marker was employed. The time lines, however, can readily be seen under magnification. *A* indicates radial curves; *B*, aortic curves.

MATERIAL

The age group from 5 to 9 years was composed of thirty-three subjects, eighteen of whom were girls. About 85 per cent were apparently normal children who were attending school; the remaining 15 per cent were hospital patients who were convalescing from acute infections such as otitis media, acute infection of the upper respiratory tract and pyelocystitis.

The age group from 10 to 14 years was comprised of thirty-seven children, twenty-one of whom were boys. Their selection was similar to that of the previous group. Observations were made on sixty-four in the age group between 15

24. Broemser, P., and Ranke, O. L.: Ueber die Messung des Schlagvolumens des Herzen auf unblutigem Wege, Ztschr. f. Biol. **90**:467, 1930.

and 19 years, of whom twenty-three were boys. Of the twenty-three, 56 per cent fell in the age period from 15 to 17 years, and of forty-one girls, 83 per cent were 18 and 19 years of age. Uniformity between the males and females is therefore lacking in this group. Of the forty-one females, approximately 81 per cent were nurses who had just entered training. The twenty-three males were all hospital patients, most of them convalescing from herniotomies and appendectomies. Several were convalescing from lobar pneumonia and other acute infections.

The age group from 20 to 24 years comprised a hundred and twenty subjects. Thirty-five were males, all medical students. The eigthy-five females were nurses. They were evenly distributed in the age period.

There were eighty-seven persons in the age group between 25 and 29 years, eighteen of whom were females. The sixty-nine males were interns, residents and senior medical students. Ninety per cent of the females were nurses, and 70 per cent of them fell in the age group from 25 to 26 years.

The age group from 30 to 39 years was comprised of seventy-seven persons, of whom seventeen were females. They were fairly well distributed throughout the ten year age period. Approximately 75 per cent were hospital and dispensary patients. They were carefully questioned and examined for the presence of cardiovascular diseases. No person suspected of having cardiovascular disease was used in this investigation. Of the 75 per cent approximately 50 per cent were patients who were being treated for peptic ulcers.

A similar situation existed in the age group from 40 to 49 years, comprising seventy-three persons. Approximately 80 per cent of these were hospital and dispensary patients. It is interesting to know that when the selection for this age group was made, a considerable number of candidates were rejected because of the presence of conditions suggesting cardiovascular involvement, such as coronary disease, hypertension and arteriosclerosis. Only 15 per cent of the persons in this group were females. The small number of females was due to the fact that a large number of the candidates were rejected on account of obesity. Obesity makes it difficult to determine accurately the length of the aortic-iliac stretch.

The same condition obtained in the age group from 50 to 59 years with fifty-eight subjects, of whom 6.5 per cent were females. With few exceptions, no one with a diastolic pressure above 90 was accepted. This rule applied to all groups. Likewise, a systolic pressure of over 150 was considered a cause for rejection.

The age group from 60 to 69 years consisted of thirty-three persons of whom five were females. In the age group from 70 to 78 years only nine cases were reported, of which one was in a woman. These were also hospital and dispensary patients who appeared to be in a fairly good state of health. None had hypertension, yet all of them had definitely thickened and palpable brachial vessels. Some tortuosity of the brachial vessels was also noted. None had palpable goose-ring brachial or radial vessels. Furthermore, in all nine cases roentgenograms of the heart taken at a distance of 6 feet (182 cm.) showed no definite cardiac enlargement.

The chief difficulty in selecting proper subjects from the older age groups appears to be that no method is available by which one can evaluate even approximately the condition of the brachioradial and aortic vessels. Arteriosclerosis is a common observation in people over 45 years of age, but the degree present varies considerably. Palpation is, after all, too crude a method to be employed in the selection of subjects. The pulse pressure gives one, at times, a notion of the degree of arteriosclerosis of the aorta in aged persons, although the finding of high pulse pressures in all persons (assuming, of course, aortic insufficiency to be ruled out) who have aortic arteriosclerosis is indeed inconstant. The size of the cardiac

shadow may be of some aid. This procedure was carried out in the age group from 70 to 79 years before the velocities of the pulse wave were recorded. However, if one accepts the work of Fahr and his associates,[25] arteriosclerosis without narrowing the lumen of the aorta presumably does not increase the work of the left ventricle, and therefore people with arteriosclerosis of the aorta, uncomplicated with other cardiovascular diseases, should not have big hearts.

While I am fully aware of the fact that the subjects selected in the older age groups, especially over 60 years may not have been altogether characteristic examples of this age group, every attempt was made to select subjects who appeared to be as normal as one is able to judge, at least as far as the cardiovascular system was concerned.

It is recognized that if measurements could be made on older persons of the higher walks of life, whose living conditions have rendered them less liable to diseases of the circulatory system and of associated diseases, a more accurate cross-section of the normal population for the particular age group might be obtained.

RESULTS AND INTERPRETATIONS [26]

In order to demonstrate the age changes in the velocity of the pulse wave, the values of the velocities of both radial and aortic pulse waves were plotted against age, and the mean curve drawn through the series of observations. The resulting graphs (figs. 2 and 3) show the age increase and dispersion or spread of the observations. The central trends of the velocity of both radial and aortic pulse waves and of the two sexes combined are illustrated by point-to-point curves drawn so as to represent the means for each age period (tables 3 and 4). The central zones of dispersion expected to enclose two thirds of the cases are drawn in according to $\pm$ 1 standard deviation (tables 6 and 7) from the mean for each age group, with the exception of the velocity of the radial pulse wave for the last age period, in which the standard error for the previous age period is projected in dotted lines. The sex differences in age trend are shown in figure 4.

The means and their standard errors for the velocity of the aortic and radial pulse waves for males, females and both sexes for each age group are given in tables 3 and 4, respectively. The standard deviations for each age period and each sex and their standard errors are given in tables 6 and 7.

With few exceptions, values for the velocity of both radial and aortic pulse waves tend to increase in successive age periods. The trend

25. Fahr, G.; Davis, J.; Kerkhof, A.; Hallock, P., and Giere, E.: Hemodynamics of Arteriosclerosis, Am. J. Physiol. **101**:376, 1932.

26. In the following discussion, for the sake of brevity, I have purposely avoided the use of the terms "arterial elasticity" and "arterial rigidity." The velocity of pulse waves is a direct function of arterial rigidity and an inverse function of arterial elasticity. It is therefore essential that one bear in mind that the terms indicating an increase of velocity of radial and aortic pulse waves imply an increase of arterial rigidity or, conversely, a decrease of arterial elasticity.

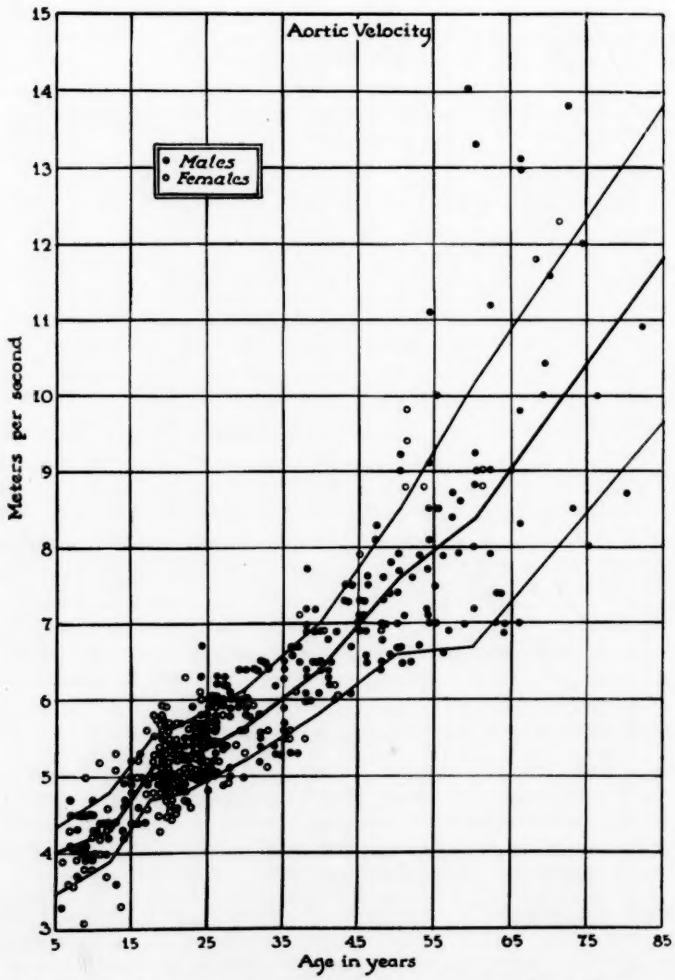

Fig. 2.—Graph showing the distribution of velocity of aortic pulse waves with age. The central trend is indicated by the point to point curve drawn into the mean velocities for five and ten year age intervals. The central zone of variability expected to contain two thirds of the individual observations is indicated by the point to point curves drawn into the standard deviation for each age interval.

is illustrated in figure 4. The differences between mean velocities for adjacent age groups appear significant (tables 8 and 9) when subjected to statistical analysis. The differences between the means of successive age groups are in this study usually more than 2 times their standard errors; i. e., they would occur by chance alone less than five times in a hundred.[27]

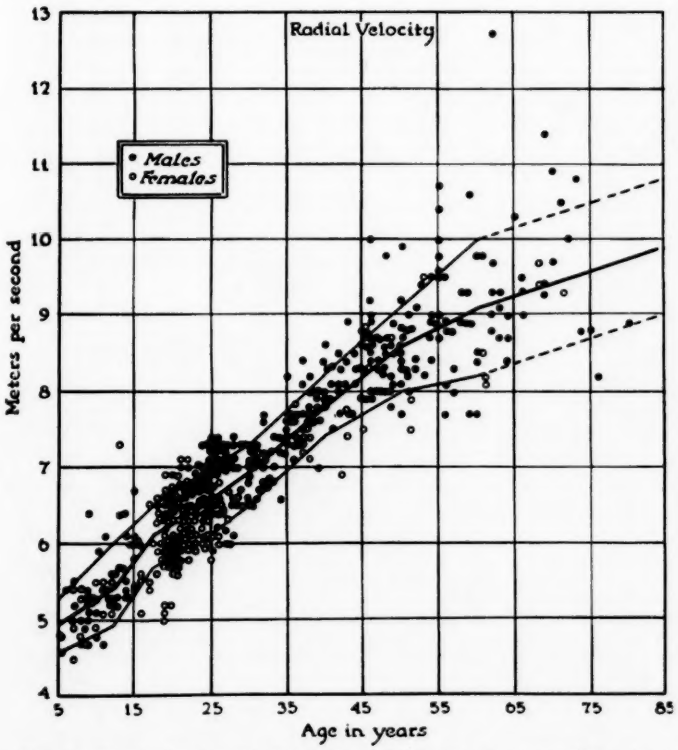

Fig. 3.—Graph showing the distribution of velocity of mean radial pulse waves with age. The central trend is indicated by the point to point curve drawn into the mean velocities for five and ten year age intervals. The central zone of variability expected to contain two thirds of the individual observations is indicated by the point to point curves drawn into the standard deviation for each age interval.

27. In ordinary statistical practices, when a difference between two means is 2 times the standard error, the difference is considered possibly significant, since such a difference would occur by chance only five times in a hundred (Dunn, H. L.: Statistical Methods in Physiology, Physiol. Rev. **9**:275, 1929). Some biometricians consider that it is wiser to use 3 times the standard error. Then, P is 0.01; i. e., difference would occur by chance only one time in a hundred.

TABLE 3.—*Age Changes in the Aortic Velocity of Both Sexes (Mean Aortic Velocity and Its Standard Error)*

Age, Years	Males		Females		Difference and Its Standard Error	Difference		Both Sexes	
	Number	Mean	Number	Mean		Standard Error	Probability Integral	Number	Mean
5 to 9	13	4.131 ± 0.147	11	4.000 ± 0.156	−0.131 ± 0.214	0.61	0.542	24	4.071 ± 0.090
10 to 14	17	4.341 ± 0.077	15	4.313 ± 0.147	−0.028 ± 0.166	0.17	0.865	32	4.328 ± 0.078
15 to 19	18	4.878 ± 0.094	39	5.228 ± 0.065	+0.350 ± 0.114	3.06	0.003	57	5.118 ± 0.058
20 to 24	31	5.352 ± 0.078	77	5.149 ± 0.043	−0.203 ± 0.089	2.28	0.021	108	5.207 ± 0.039
25 to 34	80	5.699 ± 0.048	19	5.421 ± 0.077	−0.278 ± 0.091	3.06	0.003	99	5.645 ± 0.044
35 to 44	45	6.482 ± 0.085	9	6.100 ± 0.191	−0.382 ± 0.209	1.83	0.064	54	6.419 ± 0.079
45 to 54	45	7.442 ± 0.132	7	8.371 ± 0.431	+0.929 ± 0.451	2.06	0.040	52	7.567 ± 0.134
55 to 64	27	8.419 ± 0.358	3	8.600 ± 0.306	+0.181	....	.....	30	8.437 ± 0.322
65 and over	16	10.256 ± 0.502	2	12.050 ± 0.249	+1.594	....	.....	18	10.456 ± 0.465
	292		182					474	

TABLE 4.—*Age Changes in the Radial Velocity of Both Sexes (Mean Radial Velocity and Its Standard Error)*

Age, Years	Males		Females		Difference and Its Standard Error	Difference		Both Sexes	
	Number	Mean	Number	Mean		Standard Error	Probability Integral	Number	Mean
5 to 9	17	5.176 ± 0.099	10	4.970 ± 0.099	−0.206 ± 0.140	1.47	0.142	27	5.100 ± 0.073
10 to 14	19	5.453 ± 0.098	12	5.508 ± 0.200	+0.055 ± 0.223	0.25	0.802	31	5.474 ± 0.097
15 to 19	22	6.045 ± 0.081	40	6.103 ± 0.068	+0.058 ± 0.106	0.55	0.582	62	6.082 ± 0.053
20 to 24	34	6.597 ± 0.059	82	6.261 ± 0.043	−0.336 ± 0.073	4.60	0.000	116	6.359 ± 0.039
25 to 34	93	6.930 ± 0.038	24	6.638 ± 0.075	−0.292 ± 0.084	3.47	0.000	117	6.870 ± 0.036
35 to 44	52	7.885 ± 0.053	14	7.386 ± 0.070	−0.499 ± 0.088	5.68	0.000	66	7.779 ± 0.051
45 to 54	63	8.624 ± 0.061	10	8.120 ± 0.190	−0.504 ± 0.200	2.53	0.012	73	8.555 ± 0.062
55 to 64	39	9.110 ± 0.151	3	8.600 ± 0.451	−0.510	....	.....	42	9.074 ± 0.143
65 and over	16	9.675 ± 0.683	3	9.467 ± 0.107	−0.208	....	.....	19	9.642 ± 0.573
	355		198					553	

TABLE 5.—*Age Changes in the Diastolic Blood Pressure of Both Sexes (Mean Diastolic Blood Pressure and Its Standard Error)*

Age, Years	Males		Females		Difference and Its Standard Error	Difference		Both Sexes	
	Number	Mean	Number	Mean		Standard Error	Probability Integral	Number	Mean
5 to 9	16	63.438 ± 2.916	8	56.750 ± 4.585	−6.688 ± 5.433	1.23	0.219	24	61.208 ± 2.503
10 to 14	19	65.632 ± 2.051	12	64.167 ± 2.524	−1.465 ± 3.252	0.45	0.653	31	65.065 ± 1.571
15 to 19	23	68.087 ± 2.296	47	74.511 ± 1.309	+6.424 ± 2.642	2.43	0.016	70	72.400 ± 1.205
20 to 24	36	75.361 ± 1.213	82	74.585 ± 0.872	−0.776 ± 1.494	0 52	0.603	118	75.093 ± 0.729
25 to 34	99	76.596 ± 0.759	25	73.160 ± 1.504	−3.436 ± 1.683	2.04	0.041	124	75.903 ± 0.687
35 to 44	55	82.145 ± 1.002	14	77.929 ± 2.379	−4.216 ± 2.581	1.63	0.104	69	81.290 ± 0.947
45 to 54	67	82.582 ± 1.138	9	76.000 ± 4.420	−6.582 ± 4.564	1.44	0.150	76	81.803 ± 1.145
55 to 64	41	81.927 ± 1.702	3	84.333 ± 1.702	+2.406	....	.....	44	82.091 ± 1.610
65 and over	21	82.762 ± 2.208	3	86.000 ± 1.000	+3.238	....	.....	24	83.167 ± 1.941
	377		203					580	

On the whole, the dispersion of mean values for the velocity of the aortic pulse wave (table 6) is essentially constant for males and females up to about 40 years, when the dispersion of values as measured by their standard deviations is significantly increased. The increasing variation from the mean aortic velocity that occurs in the later decades of life can best be explained on the ground that with increasing age, especially after the fortieth year, there begin to appear, in spite of the most meticulous selection of cases, the various manifestations of degenerative diseases of the cardiovascular system which may alter the elasticity of the arterial wall to various degrees. In the age span from 5 to 40 years the ranges of biologic variation from the mean velocities are approximately constant. From then on, regressive changes in the blood vessels alter the picture.

This is in accordance with Aschoff's [17] division of the life of arteries of the elastic type, especially of the aorta, into three periods: first, the ascending period, lasting until the age of 35, characterized by an equal increase of all tissue elements of the vessel wall; second, the summit between the ages of 35 to 45, without marked arterial changes; third, the descending stage, from the age of 45 on, characterized by various degenerative changes. Foster [28] showed also that elastic tissue of the aorta attains its maximum growth at the age of 35. Then regressive changes begin to occur. Arteriosclerotic changes in the aorta between the ages of 35 and 40 years are not uncommon.

When the differences between the standard deviation of the velocity of the radial pulse wave for successive age groups (table 7) are compared, particularly in males, the deviations appear to become significant at 50 years. At 55 years the difference is definitely significant. In the aorta, the difference of the standard deviations (table 6) becomes significant at 40 years of age. From this it can be concluded that the degenerative changes, at least in males, commence later in life in the medium-sized vessels than in the aorta. This corroborates the view that the wear and tear begins in the aorta.

The standard deviations for the velocities of both aortic and radial pulse waves (tables 6 and 7) in the old age period (graphically represented in charts 2 and 3) are obviously large. This may be due to the fact that the persons in this group were not true normal subjects. On the other hand, the large values indicate that there may occur in the older ages a variety of pathologic changes. The changes may so alter the condition of the aortic wall as to produce wide variations in elasticity reactions. For example, a hardening of the wall will increase the velocity of the pulse wave, whereas marked ectasia will tend to lower pulse wave velocity. Moreover, tortuosity tends to an error in measurement of

28. Foster, L. S.: Changes Occurring in the Elastic Fibers of the Aorta with Advancing Age, J. M. Research **21**:297, 1909.

TABLE 6.—*Differences Between the Standard Deviations of Aortic Velocity for Successive Age Groups*

Age, Years	Males					Females					Both Sexes				
	Number	Standard Deviation and Its Standard Error	Difference and Its Standard Error	Diff. / S.E.	Probability Integral	Number	Standard Deviation and Its Standard Error	Difference and Its Standard Error	Diff. / S.E.	Probability Integral	Number	Standard Deviation and Its Standard Error	Difference and Its Standard Error	Diff. / S.E.	Probability Integral
5- 9	13	0.362 ± 0.074	.....	....	.....	11	0.492 ± 0.110	.....	....	.....	24	0.431 ± 0.064			
10-14	17	0.309 ± 0.055	−0.053 ± 0.092	0.58	0.562	15	0.550 ± 0.104	+0.058 ± 0.151	0.38	0.704	32	0.437 ± 0.055	+0.006 ± 0.084	0.07	0.944
15-19	18	0.386 ± 0.066	+0.077 ± 0.086	0.90	0.368	39	0.399 ± 0.046	−0.151 ± 0.114	1.32	0.187	57	0.421 ± 0.040	−0.016 ± 0.068	0.24	0.810
20-24	31	0.425 ± 0.055	+0.039 ± 0.086	0.45	0.653	77	0.375 ± 0.030	−0.024 ± 0.055	0.44	0.660	108	0.395 ± 0.027	−0.026 ± 0.048	0.05	0.960
25-34	80	0.429 ± 0.034	+0.004 ± 0.065	0.06	0.952	19	0.326 ± 0.054	−0.049 ± 0.082	0.79	0.430	99	0.435 ± 0.031	+0.040 ± 0.041	0.98	0.327
35-44	45	0.567 ± 0.060	+0.138 ± 0.069	2.00	0.046	9	0.541 ± 0.135	+0.215 ± 0.145	1.48	0.139	54	0.574 ± 0.056	+0.139 ± 0.064	2.17	0.030
45-54	45	0.877 ± 0.093	+0.310 ± 0.111	2.79	0.006	7	1.055 ± 0.310	+0.514 ± 0.338	1.52	0.129	52	0.958 ± 0.095	+0.384 ± 0.110	3.49	0.000
55-64	27	1.823 ± 0.253	+0.946 ± 0.270	3.50	0.001	3	0.432	−0.623	....	.....	30	1.736 ± 0.228	+0.778 ± 0.247	3.15	0.001
65+	16	1.944 ± 0.355	+0.121 ± 0.436	0.28	0.779	2	0.249	−0.183	....	.....	18	1.916 ± 0.329	+0.180 ± 0.400	0.45	0.653

TABLE 7.—*Differences Between the Standard Deviations of Radial Velocity for Successive Age Groups*

Age, Years	Males					Females					Both Sexes				
	Number	Standard Deviation and Its Standard Error	Difference and Its Standard Error	Diff. / S.E.	Probability Integral	Number	Standard Deviation and Its Standard Error	Difference and Its Standard Error	Diff. / S.E.	Probability Integral	Number	Standard Deviation and Its Standard Error	Difference and Its Standard Error	Diff. / S.E.	Probability Integral
5- 9	17	0.397 ± 0.070	.....	....	.....	10	0.297 ± 0.070	.....	....	.....	27	0.373 ± 0.052			
10-14	19	0.417 ± 0.070	+0.020 ± 0.099	0.20	0.841	12	0.665 ± 0.142	+0.368 ± 0.158	2.33	0.018	31	0.530 ± 0.068	+0.157 ± 0.086	1.83	0.067
15-19	22	0.373 ± 0.058	−0.044 ± 0.091	0.48	0.631	40	0.425 ± 0.048	−0.240 ± 0.150	1.60	0.110	62	0.415 ± 0.038	−0.115 ± 0.078	1.47	0.141
20-24	34	0.341 ± 0.042	−0.032 ± 0.072	0.44	0.660	82	0.390 ± 0.031	−0.035 ± 0.057	0.61	0.542	116	0.414 ± 0.027	−0.001 ± 0.047	0.02	0.984
25-34	93	0.369 ± 0.027	+0.028 ± 0.050	0.56	0.575	24	0.362 ± 0.053	−0.028 ± 0.061	0.62	0.535	117	0.387 ± 0.025	−0.027 ± 0.037	0.73	0.466
35-44	52	0.382 ± 0.038	+0.013 ± 0.047	0.28	0.779	14	0.251 ± 0.049	−0.111 ± 0.072	1.54	0.124	66	0.415 ± 0.036	+0.028 ± 0.044	0.64	0.522
45-54	63	0.481 ± 0.043	+0.099 ± 0.057	1.74	0.082	10	0.569 ± 0.134	+0.318 ± 0.142	2.24	0.025	73	0.522 ± 0.044	+0.107 ± 0.057	1.88	0.060
55-64	39	0.928 ± 0.106	+0.447 ± 0.114	3.92	0.000	3	0.638	+0.069	....	.....	42	0.916 ± 0.101	+0.394 ± 0.110	3.58	0.000
65+	16	2.647 ± 0.483	+1.719 ± 0.484	3.55	0.000	3	0.152	−0.486	....	.....	19	2.431 ± 0.405	+1.515 ± 0.417	3.63	0.000

the distance traveled, and hence tends to lower the measured velocity of the pulse wave. It must also be kept in mind that persons age at varying rates after the age of 40 years, and a retardation in the rate of the aging processes leads to a lower velocity of the pulse wave. Persons whose arteries age faster have an increased velocity of the pulse wave. The wide spread of the central zone of variability (standard deviation) after the age of 40 may be due to all these factors affecting in variable degree the individual velocities of the pulse wave. For aortic velocity (table 6) the standard deviation for the age group from 45 to 54 years is 0.958. In the age group from 55 to 64 years,

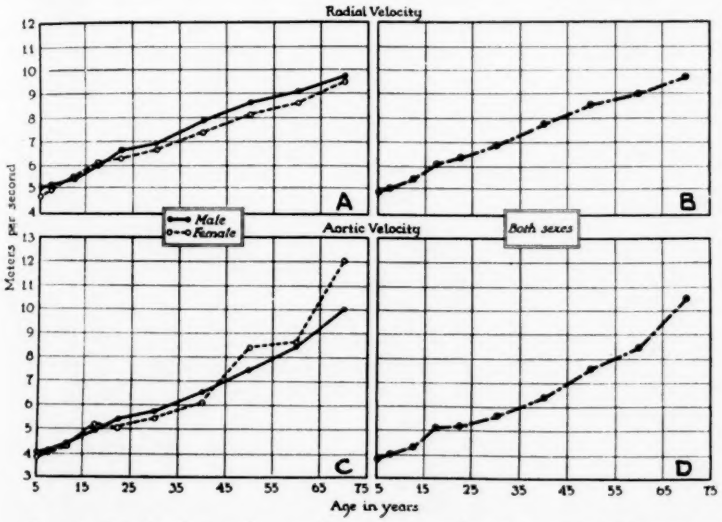

Fig. 4.—Graphs of the point to point curves, showing the progressive increase of the mean radial and aortic pulse wave velocities for males and females and for the combined sexes with age.

the value jumps to 1.736. For radial velocity (table 7) the increase is delayed ten years. It appears, therefore, that the marked degenerative changes are established in the aorta before they appear in the radial vessels. Studies of the volume elasticity coefficient of isolated vessels undoubtedly would yield considerable information in this respect and would help to explain the increasing variability found in old age. The standard deviation and its standard error for both sexes between the period from 55 to 64 is 0.916 $\pm$ 0.104, and for the age of 65 and over, 2.431 $\pm$ 0.405. The latter variations have not been presented as sigma lines in figure 3. Instead, dotted lines have been extended from points on the graph corresponding to 60 years. In all probability, two thirds

of the cases for the ages above 60 would tend to fall within the boundaries of these dotted lines, provided more cases could be recorded. It is also probable that if it were possible to obtain records from older persons representing the higher strata of life, the velocities of their pulse waves would tend to fall within the designated zone.

It is interesting to compare the age difference and standard errors for both sexes. In males the mean velocities of the radial pulse wave as compared to the mean aortic velocities, remain consistently higher until the age of 65 years is reached. After 65, the mean aortic velocity passes the mean radial velocity (tables 3 and 4). As regards females, with the exception of those in the age period from 45 to 54 years, a similar situation obtains until the age period from 55 to 64. At this period, both mean radial and mean aortic velocities become the same, 8.6 $\frac{\text{seconds}}{\text{meters}}$. After this age the mean velocity of the aortic pulse wave exceeds the mean radial velocity.

When one compares the velocities of the radial and aortic pulse waves in both sexes (tables 8 and 9) one finds that between the ages of 14 and 20, the radial and aortic velocities in females exceed these velocities in males. This phenomenon appears to be a common observation in growth curves of males and females. Scammon [29] showed from growth curves obtained in postnatal life that because females pass through most of the transition of the developmental period a little earlier than males, the physiologic age of girls is usually greater than that of boys of the same chronological age. This difference probably accounts for the increased velocity in females in the age period from 14 to 20.

Table 3 shows that there are no significant sex differences in the aortic velocities from the age of 5 to 15 years. However, after 15 years significant sex differences appear. Thus, for example, in the age period from 15 to 20 years, the difference between the two means is 3 times the standard error. Such a difference would occur by chance alone only one time in a hundred. In the age period from 35 to 44, the difference between the means is 1.83 times the standard error. This difference may be considered on the border-line of significance, for it would occur by chance alone only six times in a hundred. Likewise, in the age period between 45 and 54, the standard error of the difference may be considered possibly significant, its difference being 2 times its standard error, and the probability of it occurring by chance alone less than five in a hundred. The sex differences in the mean velocity of radial pulse waves do not become significant until the 20 year age period (table 4). After 20 years, the differences are significant; i. e., this difference is consistently greater than 2.5 times the standard error, mean-

29. Scammon, R., in Morris, H.: Human Anatomy, ed. 8, Philadelphia, P. Blakiston's Son & Co., 1925.

TABLE 8.—*Differences Between the Mean Aortic Velocity for Successive Age Groups*

Age, Years	Males					Females					Both Sexes				
	Number	Mean	Difference and Its Standard Error	Diff. / S.E.	Probability Integral	Number	Mean	Difference and Its Standard Error	Diff. / S.E.	Probability Integral	Number	Mean	Difference and Its Standard Error	Diff. / S.E.	Probability Integral
5- 9	13	4.131 ± 0.147	.....	....	.....	11	4.000 ± 0.156	.....	....	.....	24	4.071 ± 0.090			
10-14	17	4.341 ± 0.077	+0.210 ± 0.166	1.26	0.207	15	4.313 ± 0.147	+0.315 ± 0.214	1.46	0.144	32	4.328 ± 0.078	+0.257 ± 0.119	2.16	0.031
15-19	18	4.878 ± 0.094	+0.537 ± 0.122	4.40	0.000	39	5.228 ± 0.065	+0.915 ± 0.161	5.68	0.000	57	5.118 ± 0.058	+0.790 ± 0.097	8.14	0.000
20-24	31	5.352 ± 0.078	+0.474 ± 0.122	3.88	0.000	77	5.149 ± 0.043	−0.049 ± 0.078	0.63	0.529	108	5.207 ± 0.039	+0.089 ± 0.070	1.27	0.204
25-34	80	5.699 ± 0.048	+0.347 ± 0.092	3.77	0.000	19	5.421 ± 0.077	+0.272 ± 0.088	3.09	0.002	99	5.645 ± 0.044	+0.438 ± 0.059	7.42	0.000
35-44	45	6.482 ± 0.085	+0.783 ± 0.098	7.99	0.000	9	6.100 ± 0.191	+0.679 ± 0.206	3.30	0.001	54	6.419 ± 0.079	+0.774 ± 0.090	8.60	0.000
45-54	45	7.442 ± 0.132	+0.960 ± 0.016	60.00	0.000	7	8.371 ± 0.431	+2.271 ± 0.471	4.82	0.000	52	7.567 ± 0.134	+1.148 ± 0.156	7.36	0.000
55-64	27	8.419 ± 0.358	+0.977 ± 0.382	2.56	0.010	3	8.600 ± 0.306	+0.229	....	.....	30	8.437 ± 0.322	+0.870 ± 0.349	2.49	0.012
65+	16	10.256 ± 0.502	+1.837 ± 0.617	2.98	0.003	2	12.050 ± 0.249	+3.450	....	.....	18	10.456 ± 0.465	+2.019 ± 0.566	3.57	0.000

TABLE 9.—*Differences Between the Mean Radial Velocity for Successive Age Groups*

Age, Years	Males					Females					Both Sexes				
	Number	Mean	Difference and Its Standard Error	Diff. / S.E.	Probability Integral	Number	Mean	Difference and Its Standard Error	Diff. / S.E.	Probability Integral	Number	Mean	Difference and Its Standard Error	Diff. / S.E.	Probability Integral
5- 9	17	5.176 ± 0.099	.....	....	.....	10	4.970 ± 0.079	.....	....	.....	27	5.100 ± 0.073			
10-14	19	5.453 ± 0.098	+0.277 ± 0.139	1.99	0.047	12	5.508 ± 0.200	+0.538 ± 0.223	2.41	0.016	31	5.474 ± 0.097	+0.374 ± 0.121	3.09	0.002
15-19	22	6.045 ± 0.081	+0.592 ± 0.127	4.66	0.000	40	6.103 ± 0.068	+0.595 ± 0.211	2.82	0.005	62	6.082 ± 0.053	+0.608 ± 0.111	5.48	0.000
20-24	34	6.597 ± 0.059	+0.552 ± 0.100	5.52	0.000	82	6.261 ± 0.043	+0.158 ± 0.081	1.95	0.051	116	6.359 ± 0.039	+0.277 ± 0.066	4.20	0.000
25-34	93	6.930 ± 0.038	+0.333 ± 0.070	4.76	0.000	24	6.638 ± 0.075	+0.377 ± 0.086	4.38	0.000	117	6.870 ± 0.036	+0.511 ± 0.053	9.64	0.000
35-44	52	7.885 ± 0.053	+0.955 ± 0.065	14.69	0.000	14	7.386 ± 0.070	+0.748 ± 0.103	7.26	0.000	66	7.779 ± 0.051	+0.909 ± 0.062	14.66	0.000
45-54	63	8.624 ± 0.061	+0.739 ± 0.081	9.12	0.000	10	8.120 ± 0.190	+0.734 ± 0.203	3.62	0.000	73	8.555 ± 0.062	+0.776 ± 0.080	9.70	0.000
55-64	39	9.110 ± 0.151	+0.486 ± 0.163	2.98	0.003	3	8.600 ± 0.451	+0.480	....	.....	42	9.074 ± 0.143	+0.519 ± 0.156	3.33	0.001
65+	16	9.675 ± 0.683	+0.565 ± 0.699	0.81	0.418	3	9.467 ± 0.107	+0.867	....	.....	19	9.642 ± 0.573	+0.568 ± 0.591	0.96	0.337

ing that the differences in the velocity of the pulse wave would not occur oftener than once in a hundred times on the basis of chance alone.

When aortic velocities (table 8) for males between the age periods from 5 to 9 and from 10 to 14 are compared, the difference is only 1.26 times the standard error, indicating that the age change in young males is not significant. The difference in aortic velocity between the 5 to 9 and 10 to 14 year groups in females is likewise of no significance. However, the difference in the velocity of the radial pulse wave (table 9) for males between the corresponding age periods is probably significant, the difference being 1.99 times the standard error, indicating that the difference would occur through chance alone only four and a half times out of a hundred chances. For females the standard

TABLE 10.—*Comparison of the Correlation of Diastolic Blood Pressure With Aortic and Radial Velocities for Both Sexes*

	Aortic Velocity				Radial Velocity			
Age, Years	Number	r ± SE	r* / SE	P†	Number	r ± SE	r* / SE	P†
5- 9	20	0.627 ± 0.224	2.80	0.005	24	0.146 ± 0.209	0.70	0.484
10-14	32	0.312 ± 0.180	1.73	0.083	31	0.326 ± 0.183	1.78	0.075
15-19	56	0.418 ± 0.135	3.10	0.002	61	0.224 ± 0.167	1.34	0.180
20-24	107	0.071 ± 0.097	0.73	0.466	113	0.289 ± 0.094	3.07	0.002
25-34	99	0.166 ± 0.101	1.64	0.101	116	0.047 ± 0.093	0.51	0.610
35-44	53	0.231 ± 0.139	1.66	0.097	63	0.179 ± 0.127	1.41	0.159
45-54	51	0.160 ± 0.141	1.13	0.258	73	−0.072 ± 0.118	0.61	0.542
55-64	30	0.466 ± 0.186	2.51	0.012	42	0.211 ± 0.156	1.35	0.177
65 & over	18	0.270 ± 0.243	1.11	0.267	19	0.085 ± 0.236	0.36	0.719
All ages	466	0.533 ± 0.046	11.59	0.000	542	0.863 ± 0.043	20.07	0.000

* r represents the correlation coefficient and SE its standard error.
† P is the probability integral.

error of the difference between the same age period is 2.41 and therefore significant, because this difference would occur through the operation of chance alone only one and a half times in a hundred chances.

When both sexes are combined, the difference between the velocity of the radial and the aortic pulse wave for successive age groups is significant throughout except in the period from 20 to 25 years for aortic velocity.

Table 5 shows the diastolic blood pressure progressively increasing with age for males and females. Diastolic pressure is consistently lower in females than in males of corresponding ages. Significant variations between males and females appear in the age group from 15 to 19 years, in which the difference is 2.34 times the standard error. In the age group between 25 and 35, the sex difference in diastolic pressure is 2.04 times the standard error and therefore significant.

In order to determine whether any quantitative relationship exists between the velocity of the pulse wave and diastolic pressure, correlation

coefficients were determined for radial velocity and diastolic pressure and for aortic velocity and diastolic pressure. Since the correlation technic is based on the assumption of a rectilinear relation between the two variables, the velocities were plotted against diastolic pressure by means of spot diagrams. Their trends appear to be approximately rectilinear; hence this technic is applicable to this material. Table 10 shows a comparison of the correlation of the diastolic pressure with the aortic and radial velocities of the combined sexes. The correlation coefficients for all ages combined were 0.533 ± 0.046 for aortic velocity and 0.864 ± 0.043 for radial velocity. However, such correlations are of questionable value in view of the age increase in velocities and diastolic blood pressure. The factor of age increase could account for the significant correlation found. This explanation is supported by the observation that the correlations for radial velocity and diastolic pressure for the 5 and 10 year age periods are much lower than the value of the correlation coefficient for all ages combined, and that only one of the six correlations for groups after growth is complete is significant. To prove the reliability of the significance of these correlations, it would be necessary to treat the data by partial correlation when age could be held constant. The same factor of growth may account for the sex difference in correlation found by Hafkesbring and Ashman.[30] Table 10 shows in the age period of from 20 to 24 a positive correlation for radial velocity. As mentioned before, of the hundred and thirteen persons in this group, approximately 80 per cent were females. We therefore probably have a slightly positive correlation in the radial velocity, particularly in the female group. Hafkesbring and Ashman derived from approximately ninety-six observations the coefficients of correlation between subclavicular-radial and carotid-radial velocities with diastolic pressures. In both instances they found significant correlations in females, with correlation coefficients of 0.662 ± 0.056 and 0.529 ± 0.093, respectively. Significant coefficient correlations were not found in males. However, if one considers that the age spread from which their correlation coefficients were computed varied from 17 to 36 years, it appears that the correlations of these investigators are spurious, as the age changes are probably responsible for the high correlation values. Similarly, the high correlation of coefficients found by Sands in her series of eight cases must be disqualified on similar ground. It seems, therefore, that the coefficient of correlations as computed in table 10 for radial velocities between 20 to 24 years of age represents a more accurate value.

In figure 4 the effect of age on the mean velocity of the pulse wave is again graphically represented in the form of point to point curves.

30. Hafkesbring, R., and Ashman, R.: Pulse Wave Velocity in Ninety Subjects, Am. J. Physiol. **100**:89, 1932.

Figures *A* and *C* demonstrate again the increasing radial and aortic velocity in females in the age period between 12 and 20 as compared to that in males. The abrupt rise in aortic velocity at the age of 60 for both sexes may be accounted for by the fact that only a few observations were made on elderly females and that they all had high pulse wave velocities.

It is interesting to compare the mean radial velocities, from table 4, with those obtained by Bramwell, Hill and McSwiney.[22] Their series comprised seventy-four observations on both males and females varying in age from 4 to 84 years, and in pulse wave velocity from 4.7 to 8.6 meters per second. Fifty-three of their observations were made on persons below 25 years of age, while only twenty-one were made on older subjects. In this study, a total of five hundred and fifty-three observations was made on the velocities of the radial pulse wave alone.

It is obvious from table 11 that the figures on the velocity of the pulse wave are in excellent agreement, except after 45 years of age.

TABLE 11.—*Comparison of the Mean Velocities of the Radial Pulse Wave*

Age periods	5-9	10-14	15-19	20-24	25-35	36-44	45-54	55-64
Bromwell, Hill and McSwiney [22]	5.3	5.7	6.05	6.3	6.7	7.3	7.6	8.0
Hallock	5.1	5.5	6.08	6.3	6.9	7.7	8.6	9.0

The mean velocities of Bramwell and his associates from 50 years upward are based on seven observations, while the mean values in my observations are based on approximately seventy-five.

Excluding the higher age group, the table shows the close agreement obtained by the use of two different recording instruments, in one instance, the hot wire sphygmograph used by Bramwell, Hill and McSwiney, and in the other, the segment capsule apparatus employed in this study.

CONCLUSIONS

The essential factors which modify arterial elasticity in man have been discussed and the literature reviewed. The fundamental theoretical considerations from which it is possible to evaluate arterial elasticity in living man from the velocity of the pulse wave have been briefly reviewed.

A method has been described by which the transmission time of the pulse wave through the aortic iliac vessels can be determined.

Within the limitations of these data, the following conclusions are drawn:

1. There is a progressive increase of the velocities of the aortic and radial pulse waves (arterial rigidity) with age, from a value of 4.1 meters per second in midchildhood to 5.2 meters per second at 22

years of age and to 10.5 meters per second at 65 years of age for aortic velocity, and in the brachial-radial vessels in midchildhood from a value of 5.1 meters per second to 6.3 meters per second at 22 years of age and to 9.6 meters per second at 65 years of age.

2. The velocity of the radial pulse wave in males is greater than that in females, except in the age period between 10 and 19 years. During this period, the velocity of the female pulse wave slightly exceeds that of the male. This is probably due to the fact that the physiologic age of females is greater than that of males during this period of life.

3. The velocity of the aortic pulse wave in males exceeds the velocity of the aortic pulse wave in females, except in the age period between 15 and 19 years. Here again the velocity of the pulse wave in females exceeds that in males for the same reason as was stated in the preceding paragraph. The velocity of the aortic pulse wave in females exceeds that in males after the age of 45. This discrepancy is probably due to the fact that less than ten observations were made on females for each ten year interval after the age of 35.

4. The velocity of the radial pulse wave is consistently higher than that of the aortic pulse wave until the 65 year age period is reached, indicating that the transmission time of the pulse wave is more rapid through the medium-sized vessels than through the larger arteries, such as the aorta and iliac vessels.

5. The dispersion of individual values for pulse wave velocities about the mean velocity of the aortic pulse wave as measured by the standard deviation is essentially the same from 5 to 40 years, after which it increases rapidly and significantly. The dispersion of individual values about the mean radial velocity is essentially the same from 5 to 50 years, after which it increases rapidly and significantly. Thus the increase in variability in radial velocity lags ten years behind that of the aortic velocity. These findings suggest that the greater dispersion of values for pulse wave velocity is caused by pathologic changes in the blood vessels, occurring earlier in the aorta. The effect of these changes is to increase the rigidity of the aortic-iliac tubing.

6. The differences between the mean velocity of the aortic pulse wave for successive age groups of the combined sexes are all significant, except for the age period between 20 and 25 years. When the sexes are considered separately, the mean difference between the 5 to 9 and the 10 to 14 year period is not significant in either sex. Likewise, the difference in the mean pulse wave velocity is not significant between the age groups of 15 to 19 and 20 to 24 in females.

7. When the differences between the mean velocities of the radial pulse wave for successive age groups for the combined sexes are considered, they are all significant except from the age of 65 and over. When the sexes are analyzed separately, the differences between the

15 to 19 and 20 to 24 year age groups for females are on the borderline of being significant. The differences in the mean velocity for successive ages are all significant in the male.

8. Sex differences as regards the velocity of the radial pulse wave do not become significant until the second decade is reached. However, when the aortic velocity is considered, sex changes become significant at 15 years of age. In the age period between 35 and 44 years, the age change is on the border-line of significance. There is no significant difference in mean diastolic pressure between males and females, except in the age periods from 15 to 19 and from 25 to 34.

9. The correlation found in this study and that found by other workers between the diastolic blood pressure and the velocities of the radial and aortic pulse waves cannot be considered valid until more extensive analyses are made with the age factor eliminated.

Dr. Edith Boyd, from the Institute of Child Welfare and Department of Anatomy, University of Minnesota, gave valuable assistance in the preparation of the statistical work in this paper.

LATENT ACUTE RHEUMATIC CARDITIS AS DETERMINED AT AUTOPSY

ITS OCCURRENCE

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In routine autopsies at the Presbyterian Hospital, it has been occasionally observed that a patient dying of a nonrheumatic condition, and often with a history of never having suffered from rheumatic infection, nevertheless showed lesions in the heart indicative of this disease. A search through the literature failed to disclose any description of a series of such cases, although there were a few scattered references to this coincidence. Geipel [1] recorded a case of contracted kidneys showing Aschoff bodies in the myocardium and an adherent pericardium. He also stated that Aschoff mentioned a case of Askanazy's in which scanty Aschoff bodies occurred although other signs of rheumatic infection were absent. Fraenkel [2] described three cases of verrucous endocarditis (in one of which the myocardium contained numerous Aschoff bodies) in which there was no history of rheumatic infection. Pappenheimer and Von Glahn [3] recorded two cases that had no history of rheumatism in which Aschoff bodies were found in the myocardium. Swift [4] mentioned two cases in which myocardial weakness was the only clinical feature, and in which the only distinct lesions revealed at autopsy were Aschoff bodies in the myocardium. Von Glahn and Wilshusen [5] described two cases of syphilitic aortitis which had no history of rheumatic fever in which Aschoff bodies were found in the myocardium. Libman mentioned two similar cases, one occurring with typhoid fever

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1. Geipel, P.: Untersuchungen über rheumatische Myokarditis, Deutsches Arch. f. klin. Med. **85**:75, 1905.

2. Fraenkel, E.: Ueber Myocarditis Rheumatica, Beitr. z. path. Anat. u. z. allg. Path. **52**:597, 1912.

3. Von Glahn, W. C., and Pappenheimer, A. M.: Specific Lesions of Peripheral Blood Vessels in Rheumatism, Am. J. Path. **2**:235, 1926.

4. Swift, H. F.: The Pathogenesis of Rheumatic Fever, J. Exper. Med. **39**: 497, 1924.

5. Von Glahn, W. C., and Wilshusen, H. F.: Syphilitic Aortitis and Acute Rheumatic Myocarditis; Report of Two Cases, Proc. New York Path. Soc. **24**: 71, 1924.

and the other with peritonitis following perforation of a duodenal ulcer. Rhoads,[6] in a case of ulcerative endocarditis due to the meningococcus, found "a few areas which show necrotic bundles of collagen fibers surrounded by endothelial leukocytes, strongly suggesting Aschoff bodies." Clawson,[7] in eight of fifty cases of syphilitic aortitis, found in the myocardium about the aortic ring "nodular proliferative areas comparable to Aschoff bodies."

It was decided to make a more detailed study of cases of lesions of rheumatic carditis that had no clinical history of this condition, to determine how frequently they occurred and what explanation could be offered. The autopsy records of the Presbyterian Hospital for the last five years were examined. In many cases the original sections were sufficient to demonstrate the rheumatic nature of the lesions in question, but when this was doubtful, additional blocks were cut from the preserved specimens (making a total of five or six blocks for each heart). The sections were stained with hematoxylin and eosin.

During the last five years ninety-six autopsies at this hospital were considered to show rheumatic lesions of the heart. In seventeen of these cases, death was due to some apparently unrelated cause and there was no recognizable clinical indication of an active rheumatic lesion. Cases of subacute bacterial endocarditis or frank mitral stenosis were not included in this consideration. These cases are divided into two groups: those showing Aschoff bodies in the myocardium, and those in which the evidence was derived mainly from the valvular lesions. Details concerning them are summarized in the accompanying table, and two illustrative cases are described.

REPORT OF CASES

CASE 1.—*History.*—A Filipino, aged 30, had gonorrhea in 1918. On Jan. 6, 1929, he had pain in the lower dorsal part of the spine, and fever. His Wassermann reaction was 3 plus, and he received antisyphilitic treatment. His condition became worse and he was admitted to the hospital on April 6 with neoplasm of the left kidney. Tonsillitis developed. The heart was normal. Fever continued, and he died on May 16.

Autopsy.—The left kidney was replaced by neoplasm (tubular adenocarcinoma). There were metastases in the left suprarenal gland, right kidney, liver, lungs, lymph nodes (abdominal and thoracic) and vertebrae. The heart weighed 260 Gm. The mitral leaflets were somewhat thickened with numerous small, yellowish vegetations near the margin. The aortic valves were slightly thickened.

Histologic Examination.—The myocardium contained a number of Aschoff bodies. The endocardium of the right ventricle near the pulmonic valve was thickened with loose granulation tissue containing lymphocytes, plasma cells and Aschoff cells. The mitral valve was thickened by fibrous tissue containing masses

6. Rhoads, C. P.: Vegetative Endocarditis Due to the Meningococcus, Am. J. Path. **3**:623, 1927.

7. Clawson, B. J.: Aschoff Nodule, Arch. Path. **8**:664 (Oct.) 1929.

Cases Showing Lesions of Active Rheumatic Carditis Without Recognizable Clinical Symptoms

Autopsy Number	Age	Sex	Previous History of Rheumatic Fever	Clinical Condition of Heart	Autopsy Diagnosis	Evidence of Active Rheumatic Carditis
Cases Showing Aschoff Bodies in the Myocardium						
10,217	30	M	0	Normal	Tubular adenocarcinoma of kidney with metastases, Wassermann reaction +	Aschoff bodies in myocardium; endocarditis in right ventricle; thickening and vegetations of mitral leaflets
10,541	40	M		Normal	Chronic pulmonary tuberculosis; disseminated acute miliary tuberculosis	Aschoff bodies and scarring in myocardium; shortening and thickening of mitral leaflets with verrucae
10,543	34	F	0	Pericarditis (tuberculous)	Tuberculosis of tracheobronchial lymph nodes; pericardium and fallopian tubes; disseminated acute miliary tuberculosis, Wassermann reaction +	Scanty Aschoff bodies in myocardium
10,648	35	M		Normal	Chronic pulmonary tuberculosis; disseminated acute miliary tuberculosis	Scanty Aschoff bodies in myocardium
11,005	37	F	0	Normal	Pulmonary tuberculosis; disseminated acute miliary tuberculosis	Numerous Aschoff bodies in myocardium; endocarditis in left ventricle
11,041	21	M	0	Normal	Acute otitis media with mastoiditis and suppurative leptomeningitis (pneumococcus, type III)	Aschoff bodies in myocardium; mitral stenosis with verrucae
11,202	31	M	0	Normal	Chronic glomerular nephritis with edema and retinitis; tonsillitis (Staph. aureus, Strep. haemolyticus)	Aschoff bodies in myocardium; thickening of mitral leaflets with cellular infiltration and verrucae
11,228	28	M	3 years ago	Normal	Carcinoma of rectum with metastases; pelvic abscess; bacteremia (gram-negative bacillus)	Scanty Aschoff bodies in myocardium; thickening and verrucae of mitral leaflets and aortic cusps
11,252	16	F			Abortion; acute endometritis and peritonitis	Scanty Aschoff bodies in myocardium
11,375	52	M	0	Normal	Carcinoma of esophagus; acute tracheitis; arteriosclerosis	Scanty Aschoff bodies in myocardium
Cases of Rheumatic Endocarditis in Which Aschoff Bodies Were Not Found						
10,234	36	F	0	Normal	Bilateral nephrolithiasis with hydronephrosis and suppurative pyelonephritis; pulmonary tuberculosis; arteriosclerosis bacteremia (gram-negative bacillus)	Active endocarditis of mitral leaflets with verrucae
10,257	33	M	0	Normal	Lobar pneumonia (pneumococcus type I)	Active endocarditis of mitral leaflets with verrucae
10,283	48	M	0	Normal	Ulcers of esophagus and duodenum; bronchopneumonia	Active endocarditis of mitral leaflets with verrucae
10,348	33	M	0	Normal	Bronchial asthma; rupture of pleura; bilateral pneumothorax	Active endocarditis of mitral leaflets with verrucae
10,933	42	F	0	Normal	Acute suppurative peritonitis (Strep. hemolyticus) following cholecystotomy; acute suppurative pleurisy	Mitral valvulitis with verrucae; swollen collagen fibers and collections of large mononuclear cells in myocardium
11,067	63	M	0	Normal	Carcinoma of stomach; combined sclerosis of spinal cord; arteriosclerosis; acute prostatitis	Acute endocarditis of mitral leaflets with verrucae
11,184	40	M	0	Normal	Carcinoma of cecum; (excised); peritonitis; lobular pneumonia	Active endocarditis of mitral leaflets with verrucae

of small round cells and Aschoff cells; the vegetations consisted of fibrinoid material, invaded at the base by fibroblasts. No bacteria were seen in the vegetations.

CASE 2.—*History.*—A cholecystotomy was performed on a woman, aged 42, on Feb. 28, 1932. She began to have a fever on March 28. Her heart seemed normal. Empyema was discovered and drained on April 3. The pus contained streptococci. She died on April 4.

Autopsy.—There was acute suppurative pleurisy with atelectasis of the right lung. Acute suppurative peritonitis of the subphrenic region due to Streptococcus haemolyticus was found. There were bilateral hydrosalpinx and chocolate cysts on the ovaries. The heart weighed 320 Gm. The mitral valve showed nodular vegetations 0.5 mm. across at the line of closure.

Histologic Examination.—In the myocardium were a few swollen collagen fibers around the small arteries and a few large mononuclear cells. In some places the lesions closely resembled Aschoff bodies, but they were not quite typical. The nodules of the mitral valve consisted of structureless eosinophilic material; the neighboring portion of the valve was thickly infiltrated by large, irregular, elongated cells with basophilic cytoplasm and large vesicular nuclei. No bacteria were present.

COMMENT

From the table it is seen that there were ten patients with Aschoff bodies in the myocardium and seven patients with lesions of the mitral valve, histologically identical with rheumatic fever.

It is now generally agreed that the Aschoff body is a lesion specific for rheumatic fever (Swift,[4] Sacks,[8] Coombs[9] and many others). Clawson[7] is the only recent worker who has attacked this conception. It is fair to assume that in at least ten of these cases an active rheumatic infection was present at the time of death; most probably this was also the case in the other seven. During the period under review there were 1,380 autopsies at this hospital, so that these seventeen cases represent approximately 1.2 per cent of the total series. Most of the patients were between the ages of 20 and 50, particularly between 30 and 40.

The association of active rheumatic lesions with the other conditions described may, or may not, be accidental. There is the theoretical possibility that other infections may activate a latent rheumatism. On the other hand, the association may be purely fortuitous.

Analysis of the material offers little evidence for the possibility that certain other infections exert an activating influence. In the group of ten patients with rheumatic myocarditis, four died of miliary tuberculosis; five had acute nontuberculous infections of various kinds; three died of neoplasm; two gave a positive Wassermann reaction, but showed no anatomic evidence of syphilis, and one died of chronic nephritis.

8. Sacks, B.: The Pathology of Rheumatic Fever; A Critical Review, Am. Heart J. **1**:750, 1926.

9. Coombs, Carey F.: Rheumatic Heart Disease, New York, William Wood & Company, 1924.

One patient was recorded as having hemolytic streptococci in the throat. In the group of seven patients showing only valvular lesions, one had pulmonary tuberculosis; six had acute nontuberculous infections; two died of neoplasm, and three had miscellaneous conditions, including nephrolithiasis, ulcers of the esophagus and duodenum and bronchial asthma.

It would seem that these conditions were too varied to have any logical relationship with the rheumatic lesions of the heart. A large number of the patients suffered from miscellaneous infections, especially lobular pneumonia, but many were terminal conditions that occurred with equal frequency in persons who showed no postmortem evidence of rheumatic fever. The only possible exception to this conclusion is presented by the occurrence of miliary tuberculosis in four of the ten patients with rheumatic myocarditis. A search through the records of 1,380 necropsies revealed forty-four cases of miliary tuberculosis (3.2 per cent), and of the ninety-six cases of rheumatic fever four were associated with miliary tuberculosis (4.2 per cent). Conversely, during this period the rheumatic cases comprised 7.7 per cent of the total number of autopsies, and of the forty-four patients with miliary tuberculosis four (9 per cent) showed rheumatic myocarditis. Consequently, it would seem that the combination of these two conditions does not occur more commonly than might be expected from the frequency of either condition separately. In any case, the number of combined cases is too small to furnish any reliable conclusion. In this connection it may be remembered that various investigators, especially Reitter and Löwenstein [10] in recent years, claimed to have found tubercle bacilli in the blood of a large percentage of patients with rheumatic fever. But the technical methods employed by these workers were so destructively criticized by Wilson [11] that such evidence for a specific association must be regarded with caution.

The second possible explanation is that these patients died during the occurrence of a rheumatic carditis not sufficiently intense to give rise to clinical signs recognizable in the face of the symptoms of the main disease. It is well known that such a concealed carditis has taken place in persons in whom mitral stenosis developed without any clinical history of rheumatic fever. The proportion of the cases described to the total number of autopsies during this period (1.2 per cent) is not unduly high for a city in which rheumatic fever is as common as it is in New York.

10. Reitter, C., and Löwenstein, E.: Ueber den pathogenetischen Zusammenhang des akuten Gelenksrheumatismus mit der Tuberkelbazillämie, Wien. klin. Wchnschr. **45**:293, 1932.

11. Wilson, G. S.: Tuberculous Bacillaemia, Medical Research Council, Spec. Rep. Ser. 182, 1933.

SUMMARY

A series of cases is described in which evidence of rheumatic myocarditis or endocarditis was discovered at autopsy, although the presence of an active rheumatic infection had not been recognizable during life. No correlation could be established between the rheumatic disease and any one of the various pathologic conditions found. It is therefore concluded that the association is accidental. It appears that in addition to those whose condition is diagnosed clinically as rheumatic fever, 1.2 per cent of the other patients dying at the Presbyterian Hospital also have active rheumatic disease.

EFFECTS OF VACCINES AND BACTERIAL AND PARASITIC INFECTIONS ON EOSINOPHILIA IN TRICHINOUS ANIMALS

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Little is known concerning the function of the eosinophil white blood cell. Its origin and life cycle are still a matter of controversy. In man, the eosinophil normally constitutes from 1 to 4 per cent of the circulating leukocytes, and an increase is of diagnostic value in several diseases. However, it is well known that normal guinea-pigs may have as many as 30 per cent eosinophils. Brown [1] first recognized the diagnostic importance of eosinophilia in trichinosis. He recognized, however, that in severe infections the circulating eosinophils may be either absent or few.

Opie [2] produced in normal guinea-pigs with marked eosinophilia, infections with Bacillus tuberculosis, Bacillus pyocyaneus, Bacillus mucosus-capsulatus and Streptococcus pyogenes. During the course of the infection, the eosinophils disappeared not only from the blood but from the tissues. Coincident with their disappearance from the blood, there was an accumulation of eosinophils near the site of inoculation. Canon [3] observed a diminution of eosinophils in septic processes. Zappert [4] found the number of these cells reduced in pneumonia, typhoid fever and erysipelas. Similar clinical findings were reported by Türk,[5] Staubli [6] and Nägeli.[7]

Since eosinophilia is of diagnostic value in trichinosis, the question arises whether secondary infections may reduce the number of these cells to such an extent as to make the diagnosis uncertain. The studies reported in this paper were undertaken to determine (1) the number

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From the Department of Comparative Pathology, Harvard Medical School, Second and Fourth Medical Services (Harvard) and the Thorndike Memorial Laboratory, Boston City Hospital.

1. Brown, T. R.: Bull. Johns Hopkins Hosp. **8**:79, 1897.
2. Opie, E. L.: Am. J. M. Sc. **127**:988, 1904.
3. Canon, P.: Deutsche med. Wchnschr. **18**:206, 1892.
4. Zappert, J.: Ztschr. f. klin. Med. **23**:227, 1893.
5. Türk, W.: Klinische Untersuchungen über das Verhalten des Blutes bei acuten Infectionskrankheiten, Vienna, W. Braumüller, 1898.
6. Staubli, C.: Trichinosis, Wiesbaden, J. F. Bergmann, 1909.
7. Nägeli, O.: Blutkrankheiten und Blutdiagnostik, Berlin, W. de Gruyter & Company, 1919.

of eosinophil cells in the circulating blood and organs of trichinous guinea-pigs inoculated with bacteria, other parasites and vaccines, and (*2*) the relation of the number of eosinophils to the modes of encystment of Trichinella.

METHODS

Guinea-pigs obtained from one source and approximately 3 months old were used throughout this study. These animals are readily infected with Trichinella spiralis, after which their blood picture becomes similar to that of trichinosis in man, with the exception of the peculiar and little understood inclusions (Kurloff bodies) in the cytoplasm of some of the mononuclear cells.

An increase in the circulating eosinophils was induced by feeding Trichinella spiralis by two methods. In earlier experiments, guinea-pigs were starved for twenty-four hours and then forcibly fed with heavily infected, trichinous guinea-pig meat. It was sometimes difficult to find encysted larvae in the muscles of guinea-pigs infected in this manner. A more effective method used in later studies was to give an enormous number of freed living larvae by means of a pipet inserted into the esophagus. The larvae were freed from infected meat by the method described by Augustine and Theiler.[8]

Stained paraffin sections of muscles (masseter and diaphragm), lungs, liver, spleen, bone marrow and lymph nodes were made from animals killed during the course of the experiments. The number and appearance of the eosinophils were carefully noted in all the sections, and the muscles were examined for encysted parasites to determine any variation from their normal histologic aspect. The sections were stained with eosin-methylene blue, but as an aid in the studies of the muscles, the following stains were also employed: Mallory's phosphotungstic acid hematoxylin, Mallory's aniline blue stain and the latter with acid fuchsin omitted.

The following micro-organisms and vaccines were employed: the Saranac H-37 strain of tubercle bacilli, a fresh culture of Staphylococcus aureus, a strain of Trypanosoma equiperdum maintained in guinea-pigs and a triple vaccine containing typhoid and paratyphoid A and B bacilli.

As a basis for later study and comparison, the pathologic process of the muscle invaded by Trichinella is briefly described. The immediate effect on the muscle fiber in which the larvae has established itself is more or less degeneration. Within the muscle fiber the worm grows rapidly; it becomes coiled and then encysted. In the latter process, a capsule consisting of collagen fibrils laid down by the stimulated connective tissue gradually thickens and contracts into a hyaline layer, and then may become calcified. In the early stages of encystment, at each pole of the cyst there is an infiltration of leukocytes, including eosinophils. Through the entire muscle there is a diffuse cellular infiltration, and occasional eosinophils are found. There is evidence of muscular regeneration.

EXPERIMENTAL WORK

Experiment 1.—*Tubercle Bacilli.*—(*A*) A subcutaneous injection of 0.0005 Gm. of the H-37 culture of tubercle bacilli suspended in sterile saline solution was made into the right inguinal region of four guinea-pigs. Fifteen days later, trichinous meat was fed to three of these animals and to one normal guinea-pig. The remaining animal served as a tuberculous control. Charts 1 and *2* show the

8. Augustine, D. L., and Theiler, H.: Parasitology **24**:60, 1932.

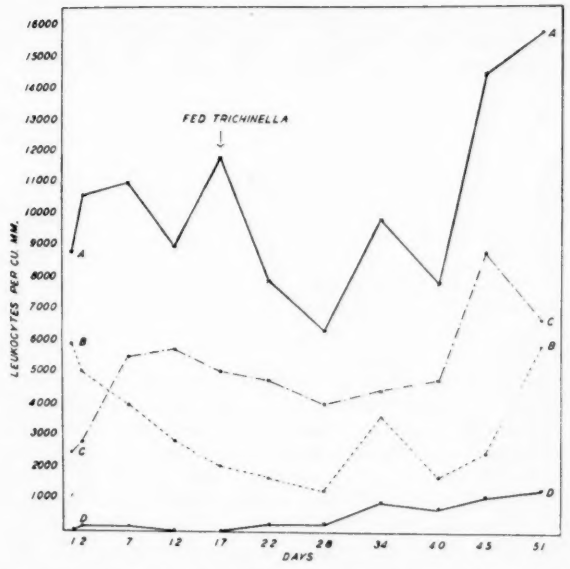


Chart 1.—The course of the white blood cells in an animal infected with Trichinella spiralis: *A*, curve for the total white blood cell count; *B*, curve for the polymorphonuclear neutrophil leukocytes; *C*, curve for the lymphocytes; *D*, curve for the eosinophil leukocytes.

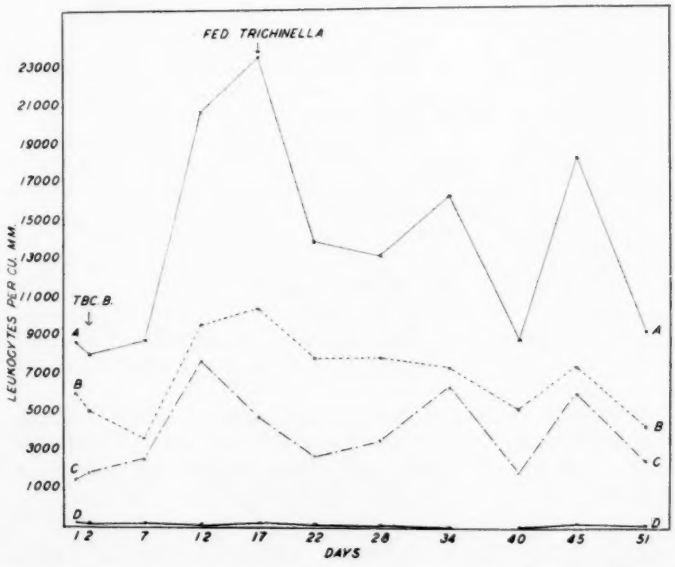


Chart 2.—The course of the white blood cells in an animal infected with B. tuberculosis and subsequently with Trichinella spiralis: *A*, curve for the total white blood cell count; *B*, curve for the polymorphonuclear neutrophil leukocytes; *C*, curve for the lymphocytes; *D*, curve for the eosinophil leukocytes.

course of the leukocytes in the trichinous animal and in one of the tuberculous-trichinous guinea-pigs. These charts show the typical results obtained in the other animals of this experiment.

In the normal trichinous pigs in this and succeeding experiments there developed an absolute increase of eosinophils, a slight decrease in polymorphonuclear leukocytes and an absolute increase in lymphocytes. In the tuberculous-trichinous pigs, there were few or no eosinophils (not more than 1.5 per cent), and the polymorphonuclear-lymphocyte ratio was in favor of the former cell, with a slight increase in the lymphocyte level as the infections progressed. There were inconstant changes in the monocytes, and the total leukocyte curve in all the animals in this experiment was variable.

The postmortem examination of animals with trichinosis alone showed an active bone marrow with many eosinophil myelocytes and leukocytes. There were large numbers of eosinophil leukocytes in the spleen and lungs, and in the cellular infiltration around the encysted parasites in the muscle. The bone marrow of the tuberculous-trichinous animals contained about the same number of eosinophil cells, but there were fewer eosinophil myelocytes and an increase of the mature type.

(*B*) In the preceding experiment, the animals were infected with tubercle bacilli before being fed with trichinous meat. For comparison, four guinea-pigs were fed with trichinous meat, and after the establishment of an eosinophilia they were inoculated with the same quantity of the H-37 strain as that used in experiment (*A*).

Chart 3 records the course taken by the white blood cells in one of the animals, which is characteristic of that in the other three. On the twentieth day after the feeding of trichinous meat, the eosinophils were 8 per cent. Tuberculosis was then superimposed on trichinosis, and the cells declined to 0.5 per cent during a period of seventeen days. There was an absolute increase in the polymorphonuclear leukocytes, with at first a decrease and later an increase in the lymphocytes. The total leukocyte count was not constant.

Postmortem examination revealed no differences in appearance of either muscle or parasites in the tuberculous-trichinous guinea-pigs as compared with trichinous guinea-pigs. However, the bone marrow, spleen and lungs of the former group had fewer eosinophils. There were none in the lymph nodes examined. The essential difference between the results of this experiment and the preceding one is that animals in which the tubercle bacillus was injected before trichinosis developed showed more eosinophils in the bone marrow, spleen and lungs than when the procedure was reversed.

Experiment 2.—*Killed Tubercle Bacilli.*—The preceding experiments were repeated, using killed instead of living tubercle bacilli. The material injected consisted of a suspension of 0.001 Gm. of the culture sterilized in an autoclave, in 1 cc. of saline solution.

The killed tubercle bacilli caused no decrease in the number of eosinophils, no appreciable changes in the total leukocyte counts and no variation in the histologic appearance of stained sections, whether the injections were made before or after the establishment of trichinosis in guinea-pigs.

Experiment 3.—*Staphylococcus Aureus.*—(*A*) Trichinous meat was fed to four guinea-pigs to induce an eosinophilia. On the twenty-first day, when the eosinophil cells had risen to a high level, a fresh culture of Staph. aureus suspended in saline solution was injected into the subcutaneous abdominal tissue of

three of the animals. The fourth animal was left as a trichinous control, while a fifth served as an uninfected control. Within twenty-four hours, there were redness and swelling at the site of the injection, with marked reduction in the number of circulating eosinophils. Two days later, there appeared an area of necrosis about 2 cm. in diameter, and the number of eosinophils continued to decline. Five days after the injection the lesions were healing, and the number of eosinophils had increased. At this time, a second inoculation of the organisms was made adjacent to the initial lesion. The tissue response was the same as in the first instance, and again the eosinophil level was reduced. As the lesions healed, the eosinophil count rose, and in two or three weeks it reached the level

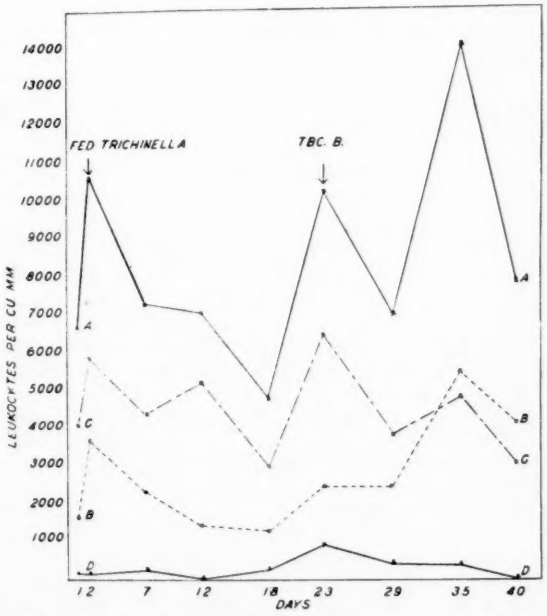


Chart 3.—The course of the white blood cells in an animal infected with Trichinella spiralis and subsequently with B. tuberculosis: *A*, curve for the total white blood cell count; *B*, curve for polymorphonuclear neutrophil leukocytes; *C*, curve for lymphocytes; *D*, curve for eosinophil leukocytes.

existing before the first injection of the staphylococci. Chart 4 illustrates the course of the white blood cells in one of the three animals which was typical of the group.

A normal animal, free from any known infection, was given two similar injections of Staph. aureus. After the first injection, there was a slight leukopenia, with a decrease in polymorphonuclear leukocytes and an increase in lymphocytes. After the second injection, however, there was definite leukocytosis, with a rise in the polymorphonuclear leukocytes and a decrease in lymphocytes.

(*B*) Effect of Heat: Certain of the animals used in this experiment were later employed to determine the effect on the eosinophil level following a rise in body temperature. The animals used included two of the guinea-pigs which had staphylococcic infections superimposed on trichinosis, the one with staphylococcic infection alone, and, as a control, another with no known infection. The animals were put into one cage and an electric heater was placed so that heat rays were directed on them. A thermometer hanging from the middle of the cage registered from 34 to 60 C. for twenty hours, the period of observation. Rectal temperatures and total and differential leukocyte counts were made just before heating, and four hours and twenty hours later. The rise in temperature in the animals varied from 1.5 to 3 C. Chart 4 records the effect of heat on the course

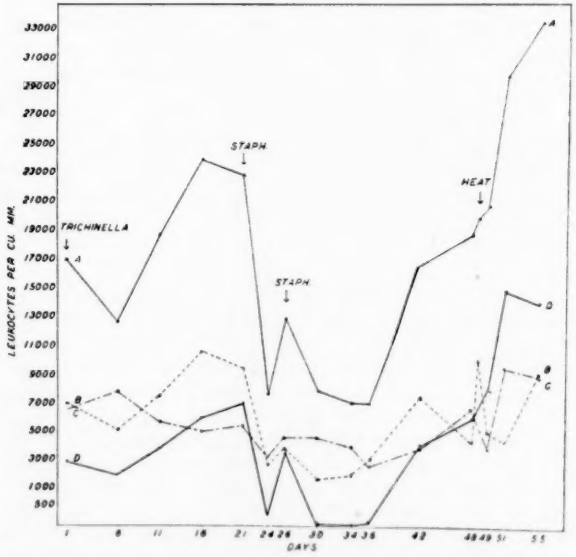


Chart 4.—The course of the white blood cells in a trichinous animal inoculated with Staph. aureus, and on the forty-eighth day of the trichinous infection subjected to a high degree of heat: *A*, curve for the total white blood cell count; *B*, curve for the polymorphonuclear neutrophil leukocytes; *C*, curve for the lymphocytes; *D*, curve for the eosinophil leukocytes.

of the leukocytes in one animal, in addition to its previous reaction to Staphylococcus. In four hours there was an increase in the eosinophils and a rise in the polymorphonuclear leukocytes. Observations at the end of forty-eight hours showed marked leukocytosis and a rise in the eosinophils. It is of interest that the animals with the combined trichinous-staphylococcic infection responded to the heat with an increase in the polymorphonuclear leukocytes and eosinophils, whereas the normal guinea-pig responded with a lymphocytosis. In the guinea-pig with the staphylococcic injection only there was a rise in the polymorpho-

Perhaps of some importance are the changes in the blood in one of the guinea-pigs which were given Staph. aureus. During the course of the trichinous infection (twenty-one days), the eosinophils failed to rise above 1 per cent. Shortly after the pyogenic infection was established, a peritonitis set in, and while the animal was in a moribund condition, large numbers of eosinophil cells made their appearance in the circulation and increased until death.

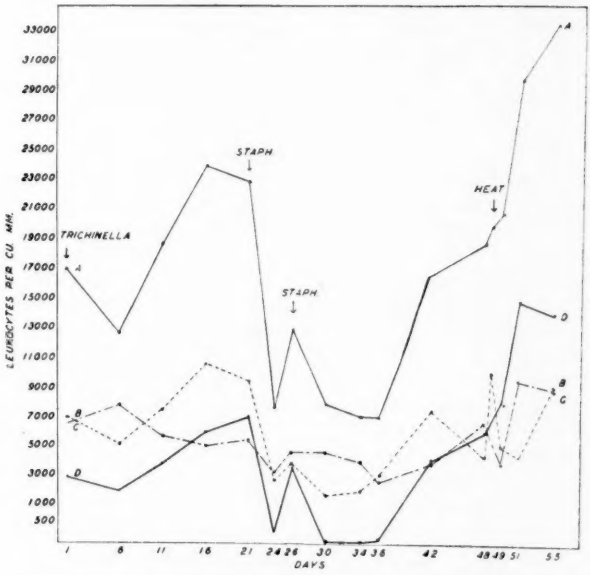
The histopathologic process in the organs of the trichinous animals was essentially the same as in the animals infected with both Trichinella and Staphylococcus. An animal which had had a trichinous infection and two staphylococcic abscesses and then been subjected to a high degree of heat (chart 4) showed one significant feature. In several sections of skeletal muscle, a septum of fibrous tissue between the muscle fibers was packed with eosinophils. This connective tissue was newly formed, as many mitotic fibroblasts were seen. The eosinophils were bilobular and elongated, as though all were moving in one direction. Nearby vessels in the tissue were dilated and showed perivascular cellular infiltration. The uninfected control which did not receive Trichinella, Staphylococcus or heat treatment possessed a high eosinophil count throughout the experiment. Sections of one of the lymph nodes presented the picture of mature eosinophil cells streaming along through the connective tissue septums. There was otherwise no evidence of an inflammatory process present in the tissue.

EXPERIMENT 4.—*Typhoid Vaccine.*—A stock preparation from the Massachusetts Antitoxin and Vaccine Laboratory containing 2,500,000,000 bacilli in 1 cc. of killed typhoid and paratyphoid A and B bacilli was used in this experiment to determine the effect of a foreign protein reaction on the blood picture and the larvae.

Little information is available in the literature as to the effects of typhoid vaccine on guinea-pigs. The same dose as used for man was used at first, i. e., 0.5 cc. the first week and 1 cc. the second and third weeks. Trichinous guinea-pig meat was fed to four animals, and simultaneously the first injection of vaccine was given in the subcutaneous abdominal tissue of each guinea-pig. The initial dose of 0.5 cc. produced no change in the leukocyte counts, nor did the animals suffer any obvious ill effects. The dose was tripled, and 1.5 cc. was injected one week later. Two of the four animals had a leukocytosis in the first twenty-four hours, and three showed a rise of the polymorphonuclear leukocytes, with a decrease of the lymphocytes and eosinophils. At the end of forty-eight hours, the eosinophils began to rise slowly, and the relationship between the polymorphonuclear cells and lymphocytes approximated that existing before injection. One week after the second injection, 3 cc. was injected into the animals with approximately the same results.

Larger quantities of vaccine were given to another animal at different intervals. Trichinous guinea-pig meat was fed, and ten days later an initial injection of 2 cc. was made. It is at this time in trichinosis that larvae are being carried in the blood stream to the muscles. Within twenty-four hours following the injection, there was a leukocytosis with a rise in the polymorphonuclear leukocytes and a decrease in lymphocytes, and the number of eosinophils dropped from 9 to 0.5 per cent. Five days after the first injection, 4 cc. of vaccine was given, resulting in a leukocytosis, but there was no change in the polymorphonuclear-lymphocyte ratio, which had returned to normal forty-eight hours after the first injection. The eosinophil level remained the same as after the first injection. After six days, or on the twenty-first day of the trichinous infection, a third injection of 4 cc. was made. The total white cell count showed a marked leuka-

(*B*) Effect of Heat: Certain of the animals used in this experiment were later employed to determine the effect on the eosinophil level following a rise in body temperature. The animals used included two of the guinea-pigs which had staphylococcic infections superimposed on trichinosis, the one with staphylococcic infection alone, and, as a control, another with no known infection. The animals were put into one cage and an electric heater was placed so that heat rays were directed on them. A thermometer hanging from the middle of the cage registered from 34 to 60 C. for twenty hours, the period of observation. Rectal temperatures and total and differential leukocyte counts were made just before heating, and four hours and twenty hours later. The rise in temperature in the animals varied from 1.5 to 3 C. Chart 4 records the effect of heat on the course



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tion of the polymorphonuclear neutrophil leukocytes and a decrease in lymphocytes. The eosinophils, which were 4 per cent before this injection, declined to zero in twenty-four hours, but rose to 7 per cent in four days, when a final dose of 4 cc. was given with the same results, except that the eosinophils did not decline.

Normal animals which were given three injections of vaccine at intervals of a week, the dosage being 0.5 cc., 1.5 cc. and 3 cc., respectively, showed a rise of the eosinophil level only following the third injection.

Microscopic examination of sections revealed only a light infestation of the muscles with Trichinella, making it impossible to arrive at any definite conclu-

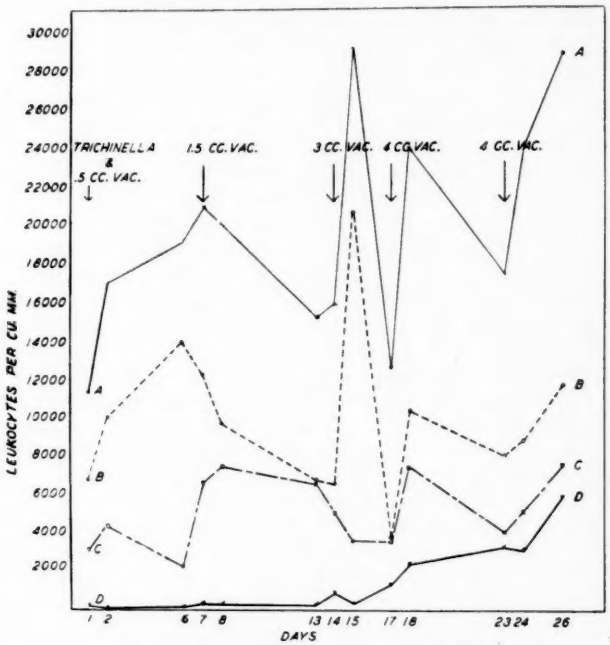

Chart. 5.—The course of the white blood cells in a trichinous animal inoculated with typhoid vaccine: *A*, curve for the total white blood cell count; *B*, curve for the polymorphonuclear neutrophil leukocytes; *C*, curve for the lymphocytes; *D*, curve for the eosinophil leukocytes.

sions as to the effect of the vaccine. For this reason, the studies were repeated on another group of animals.

To insure a heavy infection, enormous numbers of freed living larvae were fed to four animals by means of a pipet. On the same day an initial injection of 0.5 cc. of vaccine was administered. Subsequent injections of 1.5 cc., 3 cc., 4 cc. and 4 cc. were given after intervals of six, seven, three and six days, respectively. Chart 5 illustrates the course of the leukocyte counts in one of the animals typical of the group. It may be noted that there is a slight leukocytosis following each injection of typhoid vaccine, with a temporary diminution in the

eosinophils followed by an increase. There was an immediate rise in the polymorphonuclear leukocytes after each injection, while the lymphocytes tended to maintain a constant level throughout.

It is of interest that the trichinous controls lost weight, whereas all but one of the animals receiving typhoid vaccine gained in weight. Also, during the height of the disease (twentieth day) the trichinous controls were very irritable, and cried out when pressure was applied to the muscles. Those treated with vaccine showed no distress on being handled. D. L. Augustine[9] recently carried out a number of experiments to determine the therapeutic value of typhoid vaccine in trichinous guinea-pigs. His results would seem to indicate that the vaccine had no effect on the course of the infection.

The muscles of all the animals in the last group were found to be heavily infected. The muscles of the normal trichinous animals and those which were given small doses of vaccine showed more muscular degeneration, cellular infiltration and edema than those which had large doses of vaccine, as shown in chart 5. There was no difference noted in the appearance or number of eosinophils in the organs of trichinous animals which had vaccine and those which did not. It is realized that this is too small a series from which to draw any definite conclusions.

EXPERIMENT 5.—*Trypanosoma Equiperdum.*—Citrated blood containing living Trypanosoma equiperdum was injected intraperitoneally into four animals, and later a trichinous infection was produced by feeding them freed living larvae. In another group of four animals, the procedure was reversed, with the trichinosis preceding the trypanosomiasis. Trypanosomes alone were given to two animals which served as controls.

In the controls, given trypanosomes alone, the total leukocyte count decreased during the first five days after infection, and then showed an elevation, but it did not return to the initial level. There was a decline in the polymorphonuclear neutrophils and eosinophils, while the lymphocytes consisting mostly of young forms increased.

Animals which were first infected with Trypanosoma and then fed Trichinella failed to show any rise in eosinophils. At first there was a leukopenia, but following the feeding of Trichinella there was a slight rise in the total white blood count. Trichinous guinea-pigs with an eosinophilia showed after the injection of trypanosomes a decline in the eosinophil cells and a leukopenia, but no essential change in the neutrophilic polymorphonuclear-lymphocyte ratio.

One control died as a result of a ruptured spleen. All the organs were markedly blanched. The bone marrow showed many mitotic figures, but only rare eosinophils. No eosinophils were found in the spleen or liver, but many were present in the alveolar capillaries and bronchi of the lungs.

The muscles of all the animals with the combined trypanosomiasis and trichinosis were heavily infested with Trichinella. There was a tendency toward less muscular destruction, cellular infiltration and edema than in the animals infected with Trichinella only. The bone marrow, spleen, liver and muscles showed the same appearance and number of eosinophil cells as in normal trichinous controls, but there were fewer eosinophils in the lungs.

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9. Augustine, D. L.: Personal communication to the author.

COMMENT

These observations confirm the experimental work of Opie [2] and Josey and Lawrence,[10] and the clinical observations of other physicians, that acute infections cause a diminution in the number of the eosinophilic leukocytes in the peripheral blood. Previous investigations were made on normal guinea-pigs. In this study, an induced eosinophilia with a parasitic infection (Trichinella spiralis) responded similarly to secondary infection. Josey and Lawrence [10] reported that both living and killed Bacillus coli injected into normal guinea-pigs caused a reduction in eosinophils. In this study, it was found that when virulent tubercle bacilli were injected into guinea-pigs the result was a diminution of the number of eosinophils, but that killed tubercle bacilli ten times the dose of the virulent organisms exerted no influence on the eosinophil level in normal and parasite-infested animals. With repeated injections of typhoid vaccine, there was not a reduction in the eosinophils, but the percentage steadily increased. This is probably due to the fact that the animals were sensitized to the protein injected. Biggart [11] observed a similar response with repeated injections of protein solutions containing peptone, egg albumin, casein and horse serum.

A histologic examination of the tissues of trichinous animals, and those with trichinosis plus a secondary infection, revealed eosinophil cells included in the cellular infiltration around the parasites in the muscle fibers. However, this observation was not constant. This may have led Opie [12] to state that in this respect trichinosis of guinea-pigs differs from that of the human subject, as he was unable to find eosinophil cells around degenerate muscle fibers at any stage in the disease.

Animals with tuberculosis or trypanosomiasis superimposed on a trichinous infection had not only a diminution in the number of eosinophil cells in the peripheral blood, but also less evidence of muscular destruction, edema of the muscle fibers and cellular infiltration around the encysted parasites than animals with trichinosis alone. An explanation of the foregoing observation may possibly be based on immunologic principles. The cellular infiltration, edema of muscle fibers and degeneration illustrate the animal's resistance to the invading parasites. I have observed with Augustine that if trichinous animals are given a second infection with Trichinella spiralis, the cellular infiltration around the young parasites is increased considerably. Many of the larvae are outside the muscle fibers, and appear to be undergoing destruction due to the attacking leukocytes. Owing to the first infection, the animals have developed an immunity to the invading parasite, and encystment is more difficult in a second infection. Observations in this

10. Josey, A. I., and Lawrence, J. S.: Folia haemat. **48**:323, 1932.
11. Biggart, J. H.: J. Path. & Bact. **35**:799, 1932.
12. Opie, E. L.: Am. J. M. Sc. **127**:217, 1904.

study of tissue reaction may be further evidence that an immunity to Trichinella spiralis is developed following the primary invasion of the larvae. Ducas [13] and later McCoy [14] showed that in rats infected a second time with Trichinella spiralis fewer worms developed in the intestinal tract. In the trichinous animals with tuberculosis or trypanosomiasis, there was lessened reaction in the muscles owing to the fact that the animal's powers of resistance were devoted not only to resisting the invading larvae, but probably, primarily, to the more virulent and commonly fatal infections.

I am unable to explain satisfactorily the lessened cellular infiltration, edema and muscular destruction in trichinous animals receiving large doses of typhoid vaccine. A general reaction to the vaccine is indicated by the fact that each injection was followed by a leukocytosis. These animals gained in weight and were less irritable than normal trichinous controls during the height of the disease.

Further study of the tissues of all the animals revealed accumulations of eosinophil cells. They were found in abundance in the lungs of the normal controls, the trichinous animals and those with trichinosis and a secondary infection. They were seen in the capillaries, within the alveoli, especially grouped around and in the walls of the smaller bronchi and in partially destroyed cells in the lumens. This was also observed by Opie.[12] In a study of tissues from various parts of the body, this was the only evidence of destruction of these cells. Is it possible that after they have served their purpose they are excreted through the respiratory tract? Or, more pertinent, are they serving some purpose in being excreted there? It is well known that in patients with asthma, eosinophil cells are often found with ease in the sputum. I studied by ordinary methods the urine and stools of two patients with trichinosis having an eosinophilia of more than 50 per cent, and failed to find any evidence of excretion of the cells through these channels. However, Healy and his associates [15] recently observed eosinophils in the stools of a patient with gastro-intestinal allergy.

In many of the animals, an accumulation of large numbers of eosinophil cells was found in connective tissue, especially in the lymph nodes, spleen, muscles and peribronchial tissue. Opie [12] and Kanthack and Hardy [16] reported similar observations. Sometimes this connective tissue was newly formed, as mitotic figures were present. Similar observations led some of the earlier workers to believe that the eosinophil cell had its origin in connective tissue cells. Before the function

13. Ducas, R. L.: L'immunité dans la trichinose, Paris, Jouve & Cie, 1921.

14. McCoy, O. R.: Am. J. Hyg. **14**:484, 1931.

15. Healy, James C.; Gallison, Davis T., and Brudno, James: New England J. Med. **210**:123, 1934.

16. Kanthack, A. A., and Hardy, W. B.: J. Physiol. **17**:81, 1894.

of the eosinophil is fully understood, this relationship will have to be explained.

It is often stated that in certain diseases, for example scarlet fever and tuberculosis, eosinophil cells are present in the blood, mostly in the afebrile stages. In subjecting trichinous guinea-pigs to a high degree of heat so that their body temperatures were raised, the number of eosinophils, instead of being diminished, was elevated. Realizing that the number of animals studied is too small for definite conclusions, it would appear that it is not the temperature per se which causes a reduction of these cells, but rather something associated with the cause of the rise in temperature.

Throughout all the experiments, the animals were weighed periodically to see if there was a relationship between the number of circulating eosinophil leukocytes and the body weight. Opie [12] concluded that there is a direct relationship in the guinea-pig, and stated that an increase in weight is accompanied by a fall in the proportion of eosinophils, while a decrease in weight is accompanied by an increase in the number of the cells. In analyzing the weights and leukocyte counts of fifty animals, I could find no constant relationship.

While conducting this study, Dr. K. J. Thompson called my attention to a patient in the Fourth Medical Service of the Boston City Hospital, who had trichinosis complicated by a pyogenic infection. The summary of the case is as follows:

REPORT OF A CASE

A. P., a youth, aged 19, single, entered the hospital complaining of diarrhea of one week's duration and pains in the left leg. He had had intermittent chills and fever for one day, with no diarrhea. His temperature on entry was 103 F., and his pulse rate, 110. Essential positive findings on physical examination included a slightly enlarged and tender spleen, small maculopapular red blotches consistent with "rose spots," over the abdomen, open and infected ulcers of one week's duration on both feet, and soreness on palpation in the left popliteal region. A provisional diagnosis was made of typhoid fever. There was a slight leukocytosis, and no eosinophils were found in the blood. The pyogenic lesions on the feet were treated immediately on entry, and in four days were definitely improved. At this time the eosinophils were 6 per cent, and a diagnosis was made of trichinosis, which was verified by positive skin and precipitin tests. Later a biopsy of muscle revealed Trichinella.

What mechanism is involved in the diminution of the circulating eosinophil leukocytes in trichinous animals which are given a secondary infection? An endeavor was made to answer this by a study of the bone marrow. Evidence appears to favor the opinion that all eosinophil leukocytes have their origin in the bone marrow. In the preceding experiments, no evidence was found that with the decline of eosinophil cells in the peripheral blood, there was as marked a reduction in the marrow. In the secondarily infected animals, there were fewer eosinophil

myelocytes and evidence of a lessened production, but the total number of eosinophil cells was approximately the same as in trichinous animals without complications. It appears, then, that eosinophil cells are always present in the bone marrow, ready for entrance into the peripheral blood under certain influences. In the case of trichinosis, these cells enter the blood stream at a time when the larvae invade the muscles. Whether this is due to toxins liberated by the larvae at this stage or to the absorption of destroyed muscle is difficult to state, but whatever the mechanism, it is probably related to protein metabolism. If a trichinous animal is given a secondary infection, as with staphylococci, there is more of an immediate need for another type of leukocyte. But as soon as the acute stage of the pyogenic infection has passed, the eosinophil leukocyte again enters into the circulation. Previous observations on the reactions in the muscles and these hematopoietic responses indicate how appropriately these mechanisms of the body respond to various types of invasion.

SUMMARY

1. The number of circulating eosinophil leukocytes in animals infected with Trichinella spiralis was reduced following infection with B. tuberculosis, Staph. aureus and Trypanosoma equiperdum. Animals which had received repeated injections of typhoid vaccine responded with a rise in the eosinophil level. No change was noted following the injection of heat-killed tubercle bacilli.

2. Studies of the bone marrows from the same animals did not reveal a corresponding decrease in the number of eosinophil cells.

3. Trichinous animals having a superimposed infection of tuberculosis or trypanosomiasis had less reaction around the encysted parasites in the muscle than the control animals. Trichinous animals inoculated with typhoid vaccine showed similar changes in the muscles.

4. Trichinous animals subjected to a high level of dry heat responded with an absolute rise in the circulating eosinophilic leukocytes.

5. No relationship was found between the weights of animals and the level of eosinophilic leukocytes in the peripheral blood.

6. The number of circulating eosinophil cells did not appear to be related to the mode of encystment of Trichinella spiralis in the muscle.

ORAL AND DUODENAL ADMINISTRATION OF SINGLE LARGE DOSES OF PURE THYROXINE

COMPARISON OF CALORIGENIC EFFECTS WITH THOSE OF MONOSODIUM THYROXINE AND THYROXINE IN ALKALINE SOLUTION

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We have shown that single large doses of thyroxine in alkaline solution have about 63 per cent as much effect when administered by mouth as when given intravenously.[1] However, the oral administration of the monosodium salt in tablet form produces only about 25 per cent as much effect as the intravenous administration of thyroxine in alkaline solution, when the comparison is made on the basis of the daily doses of the two substances required for maintenance of the basal metabolism of patients with myxedema at the normal level.[2] As a continuation of our study of the calorigenic action of various forms of thyroxine we have now determined the effects of administering single large doses of pure thyroxine both by mouth and directly into the duodenum, and have compared them with the effects of oral administration of single large doses of monosodium thyroxine in tablet form and of thyroxine in alkaline solution.

From the Department of Medicine, Rush Medical College, and the Presbyterian and Cook County hospitals.

1. (*a*) Thompson, W. O.; Thompson, P. K.; Dickie, L. F. N., and Alper, J. M.: Effect of Alkali on the Absorption of Thyroxine from the Gastro-Intestinal Tract, Arch. Int. Med. **52**:809 (Nov.) 1933. (*b*) Thompson, W. O.; Thompson, P. K.; Taylor, S. G., III; Alper, J. M., and Dickie, L. F. N.: The Effect of Various Compounds of Thyroxine on the Basal Metabolism, Endocrinology **18**:228, 1934.

2. (*a*) Thompson, W. O.; Thompson, P. K., and Dickie, L. F. N.: Monosodium Thyroxine, Desiccated Thyroid and an Impure Sodium Salt of Thyroxine: Comparison of Their Effects When Administered Orally with the Effect of Thyroxine in Alkaline Solution Injected Intravenously, Arch. Int. Med. **52**: 576 (Oct.) 1933. (*b*) Thompson, W. O.; McLellan, L. L.; Thompson, P. K., and Dickie, L. F. N.: The Rates of Utilization of Thyroxine and of Desiccated Thyroid in Man: The Relation Between the Iodine in Desiccated Thyroid and in Thyroxine, J. Clin. Investigation **12**:235, 1933.

METHOD

As in the previous parts of this study we used only patients with definite myxedema, because of their greater response to all kinds of thyroid preparations. Five patients were used.[3] In the first patient the myxedema was spontaneous: in the others it followed a subtotal thyroidectomy for exophthalmic goiter. The fourth patient also suffered from a mild postoperative tetany which caused practically no symptoms throughout the period of observation, during which time her serum calcium was about 8 mg. per hundred cubic centimeters. The data on the third patient are not satisfactory because of irregular breathing, and his metabolic levels can only be approximated. The Sanborn-Benedict, Sanborn-Grafic and Benedict-Roth machines were used in making the determinations of the basal metabolic rate, and Aub-DuBois standards were used in the calculations. In computing the number of calories (excess calories) produced by a given dose of thyroxine, we have used a method described in previous communications.[4] Since the number of calories produced in excess of the basal rate is probably greater at higher than at lower levels of metabolism and since the method used takes into account only the basal calories, the actual excess calories recorded must be regarded as approximations and only of comparative and not absolute value. As the modes of administration of the various substances may be of importance, we have given them in detail in the legends.

All thyroxine used was crystalline synthetic thyroxine except the monosodium salt, which was in the form of tablets, each of which contained 1.03 mg. of the salt. Kendall[5] said that when thyroxine is dissolved in an excess of sodium hydroxide the disodium salt is formed. The monosodium salt is only slightly soluble in distilled water, whereas the disodium salt is said to be soluble to the extent of 4 per cent.[5] In all patients on whom data are reported here and elsewhere the alkaline solution of thyroxine (presumably the disodium salt) was prepared for oral administration by treating synthetic thyroxine with a weak solution of sodium hydroxide, except in the case of the first of two administrations of this substance to the second patient, when it was prepared by treating tablets of the monosodium salt in the same way.

For duodenal administration of thyroxine the position of the Rehfuss tube was always checked by fluoroscopy, the typical curve of the tube as it passed from the stomach into the duodenum leaving no doubt about its position. In addition, in the first patient a small amount of barium sulphate was injected through the tube the night preceding the administration of thyroxine and again about four and one-half hours preceding it. At the time of administration practically all the barium seemed to be in the ascending and transverse colons. In

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3. All the data on the first patient and about two thirds of the data on the second patient were collected at the Cook County Hospital, and the remainder at the Rush Medical College and Presbyterian Hospital. Dr. Frederick Tice and Dr. Karl A. Meyer permitted us to collect data on the patients in the Cook County Hospital.

4. Thompson, W. O.; Thompson, P. K.; Brailey, A. G., and Cohen, A. C.: The Calorigenetic Action of Thyroxin at Different Levels of Basal Metabolism in Myxedema, J. Clin. Investigation **7**:437, 1929. Thompson, Thompson, Dickie and Alper.[10]

5. Kendall, E. C.: Thyroxine, New York, The Chemical Catalog Company, Inc., 1929.

the third patient a small amount of barium sulphate was injected through the tube twenty minutes before the administration of thyroxine.

The data are recorded in charts 1 to 6 and are summarized in table 1. It may be noted that in patients 1, 4 and 5 the oral adminis-

DATA

TABLE 1.—*Comparison of Effects of Administering Thyroxine in Various Forms by Mouth and Duodenum to Five Patients with Myxedema*

Patient	Medication	Total Iodine Content of Substance Used, Mg.	Basal Metabolic Rate Before Medication; per Cent Normal	Level to Which Basal Metabolic Rate Rose, per Cent Normal	Change in Basal Metabolic Rate, Points	Length of Time Basal Metabolic Rate Was Affected, Days	Number of Squares in Graph	Number of Excess Calories Produced
1	40 mg. pure synthetic thyroxine suspended in distilled water, by duodenum	26.0	—39	—40	—1	..	...	.....
	100 mg. pure synthetic thyroxine suspended in distilled water, by mouth	65.0	—40	—35	5	7	...	.....
2	10 mg. synthetic thyroxine in the form of its monosodium salt (tablets), by mouth	6.5	—37	—22	15	50	426	6,485
	10 mg. synthetic thyroxine in the form of its monosodium salt (tablets) treated with an alkaline solution	6.5	—34	—17	17	52	580	8,910
	50 mg. pure synthetic thyroxine suspended in distilled water, by duodenum	32.5	—32	—29	3	15	31	485
	10 mg. pure synthetic thyroxine in alkaline solution, by mouth	6.5	—34	—10	24	62	837	13,025
3	10 mg. pure synthetic thyroxine suspended in distilled water, by duodenum	6.5	—18	—14	4	..	...	.....
	10 mg. synthetic thyroxine in the form of its monosodium salt (tablets), by mouth	6.5	—17	—11	6	24	...	.....
4	30 mg. pure synthetic thyroxine suspended in distilled water, by mouth	19.5	—20	—18	2	6	9	130
	30 mg. synthetic thyroxine in the form of its monosodium salt (tablets), by mouth	19.5	—22	—12	10	26	165	2,395
	10 mg. pure synthetic thyroxine in alkaline solution, by mouth	6.5	—22	— 7	15	..	...	.....
5	40 mg. pure synthetic thyroxine suspended in distilled water, by mouth	26.0	—28	—24	4	..	...	.....
	40 mg. synthetic thyroxine in the form of its monosodium salt (tablets), by mouth	26.0	—28	—20	8	..	...	.....

tration of 100, 30 and 40 mg., respectively, of pure thyroxine did not produce a clearcut change in the basal metabolic rate. There is some question of a change in the first patient following the administration of 100 mg. In this instance the basal metabolic rate rose from minus 40 to minus 35 per cent, and was back at its prethyroxine level in seven days. However, this change was too slight to be considered definite.

It should perhaps be regarded more seriously because subsequently the prolonged daily oral administration of large doses of pure thyroxine to the same patient raised the basal metabolic rate to normal, as will be reported later.

It may also be noted that in the first three patients, single doses of 40, 50 and 10 mg., respectively, of pure thyroxine, when administered directly into the duodenum, were likewise without a definite effect on the basal metabolic rate.

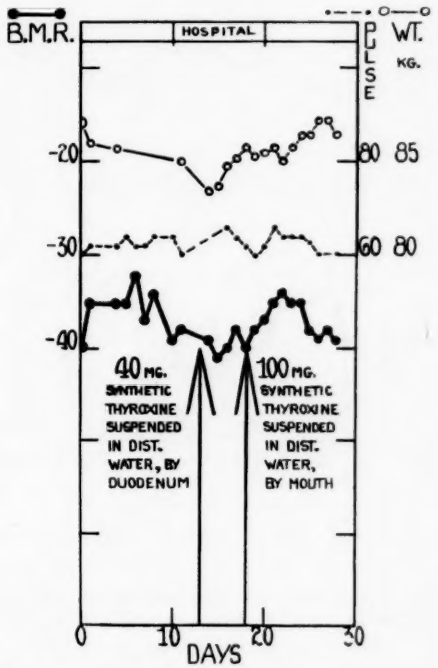


Chart 1 (Mrs. P. S.; height, 166 cm.; age 44).—The duodenal administration of 40 mg. of pure synthetic thyroxine was made at 3 p. m. on Feb. 9, 1933, through a Rehfuss tube which was in the duodenum about 10 cm. The thyroxine was administered in a suspension of lukewarm distilled water, a total of 375 cc. being used, largely for rinsing. The patient had had lunch three hours before the administration. The oral administration of 100 mg. of pure synthetic thyroxine was made at 7:50 p. m. on Feb. 14, 1933, in a suspension of lukewarm distilled water, a total of 500 cc. being used, largely for rinsing. The patient had received her usual three meals during the day, the evening meal having been served about 5 p. m.

To the second, third, fourth and fifth patients, single doses of 10, 10, 30, and 40 mg., respectively, of thyroxine were administered by

mouth in the form of the monosodium salt. A definite, though slight, increase in the basal metabolic rate and some clinical improvement were noted in all. One of the first things apparent was that the effect in the second patient was greater than in the other three. Thus, in her, 10.3 mg. of the monosodium salt raised the basal metabolic rate from minus 37 to minus 22 per cent, and resulted in the production of

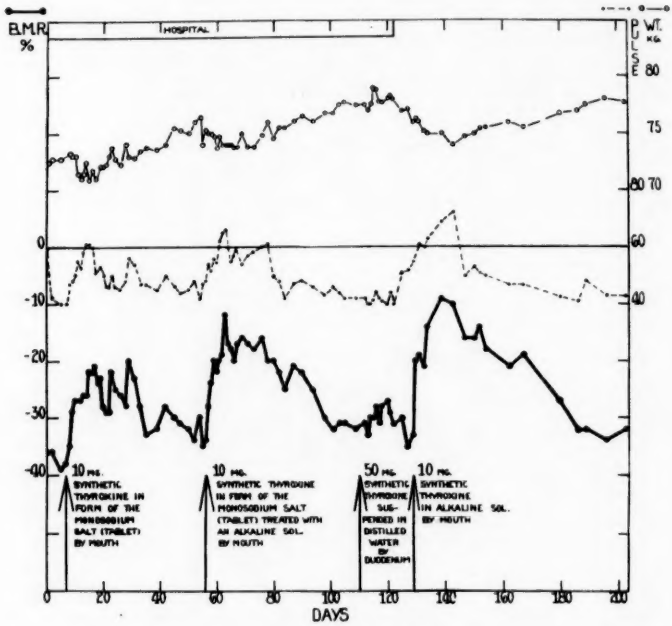


Chart 2 (Mrs. B. G.; height, 158 cm.; age 38).—The oral administration of 10 mg. of thyroxine in the form of its monosodium salt was made at 6 p. m. on Nov. 11, 1932, about 100 cc. of distilled water being used to wash down the tablets. The patient had had her usual three meals during the day, supper having been served at 5 p. m. The oral administration of 10 mg. of thyroxine in the form of its monosodium salt, which had been treated with an alkaline solution, was made at 4:20 p. m. on Dec. 30, 1932, a total of 5 drops of a 10 per cent solution of sodium hydroxide being used for solution and rinsing. In addition, 15 grains (0.96 Gm.) of sodium bicarbonate was given in solution at 4:15 p. m. and 10 grains (0.64 Gm.) in solution at 4:25 p. m. A total of 200 cc. of distilled water was used for all purposes. The patient had had breakfast, and had lunch at 12 noon. The duodenal administration of 50 mg. of pure synthetic thyroxine was made through a Rehfuss tube at 4:30 p. m. on Feb. 22, 1933, suspended in distilled water, a total of 400 cc. of water being used, largely for rinsing. The patient had not eaten since the preceding night. The oral administration of 10 mg. of synthetic thyroxine in alkaline solution was made at 1:20 p. m. on March 13, 1933, a total of 4 drops of 10 per cent solution of sodium hydroxide and 250 cc. of distilled water being used for solution and rinsing. The patient had received no food since 5:30 p. m. the preceding day.

6,485 excess calories, whereas in the third patient, the same dose caused an increase of only from minus 17 to minus 11 per cent; in the fourth patient 30 mg. caused an increase only from minus 22 to minus 12 per cent and the production of 2,395 excess calories, and in the fifth patient 40 mg. caused an increase of only from minus 28 to minus 20 per cent. The reason for this variation in effect is not clear.

To the second and fourth patients we administered 10 mg. of thyroxine by mouth in alkaline solution. As pointed out, it was

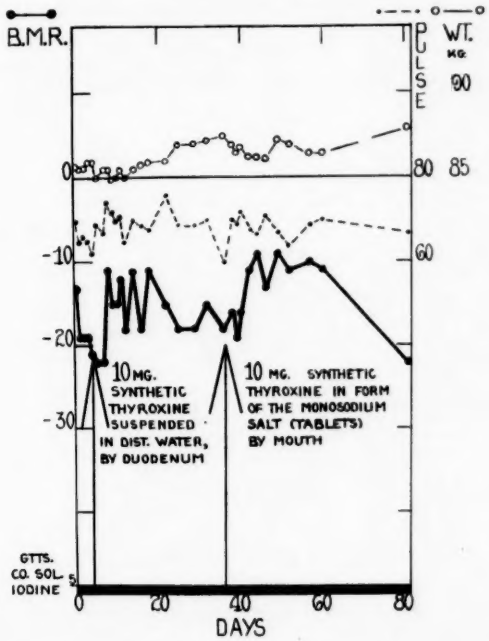


Chart 3 (Mr. A. H.; height, 181 cm.; age 47).—The duodenal administration of 10 mg. of pure synthetic thyroxine was made at 6:50 p. m. on Jan. 20, 1933, through a Rehfuss tube, in a suspension of distilled water, a total of 450 cc. of water being used, largely for rinsing. The patient had not eaten since 8 p. m. the preceding day, except for an Ewald meal six hours before the administration, most of which was aspirated; but he ate dinner twenty minutes after the administration. The oral administration of 10 mg. of thyroxine in the form of its monosodium salt (10.3 mg. of the salt) was made at 1 p. m. on Feb. 21, 1933, in the form of tablets which were washed down with 450 cc. of distilled water. The patient had had no breakfast or lunch.

administered twice in this form to the second patient. The first time the alkaline solution was prepared by treating tablets of the monosodium salt with sodium hydroxide and the second time by dissolving pure

crystalline thyroxine in an excess of sodium hydroxide. For some reason not clear at present, when the tablets were used the effect was less than when crystalline thyroxine was dissolved in an excess of sodium hydroxide. When the tablets were treated with sodium hydroxide some powder remained undissolved, which it was thought represented the base used in making the tablets (66 per cent sugar of milk and 33 per cent arrowroot starch) because the monosodium salt is soluble in a weak solution of sodium hydroxide. It is possible, however, that some of the monosodium salt was undissolved. When the tablets were used, a single dose containing the equivalent of 10 mg.

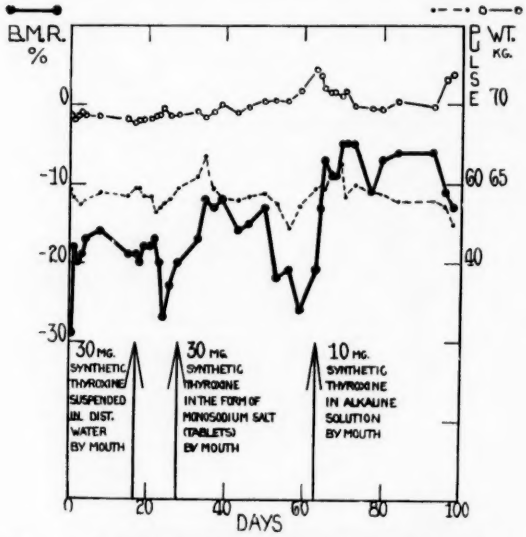


Chart 4 (Mrs. C. B.; height, 152 cm.; age 33).—The dose of 30 mg. of pure synthetic thyroxine was given by mouth at 1:50 p. m. on Jan. 26, 1933, the patient having fasted since the night before. It was given suspended in lukewarm distilled water, a total of 400 cc. being used, largely for rinsing. The dose of 30 mg. of thyroxine in the form of its monosodium salt (30.9 mg. of the salt) was given by mouth in tablet form at 12:30 p. m. on Feb. 6, 1933, the patient having had no food after 6 p. m. on February 5. The tablets were washed down with 400 cc. of lukewarm distilled water. The oral administration of 10 mg. of pure synthetic thyroxine in alkaline solution was made at 1:10 p. m. on March 13, 1933, using 4 drops of 10 per cent solution of sodium hydroxide and a total of 250 cc. of distilled water for solution and rinsing. The patient had been fasting since the preceding night.

of thyroxine increased the basal metabolic rate from minus 34 to minus 17 per cent and caused 8,910 excess calories to be produced. When the same dose of pure crystalline thyroxine was used after

dissolving it in an excess of sodium hydroxide, the basal metabolic rate was increased from minus 34 to minus 10 per cent and 13,025 excess calories were produced. In the fourth patient the administration of 10 mg. of thyroxine in alkaline solution caused an increase in basal metabolic rate from minus 22 to minus 7 per cent. Thus, in the fourth patient, 10 mg. of thyroxine in alkaline solution produced one and a half times as much effect as 30 mg. in the form of the monosodium salt, and about twice as much effect as 40 mg. in the form of the monosodium salt in the fifth patient.

Data reported here and elsewhere showing the effects of administering single large doses of thyroxine in various forms and by various

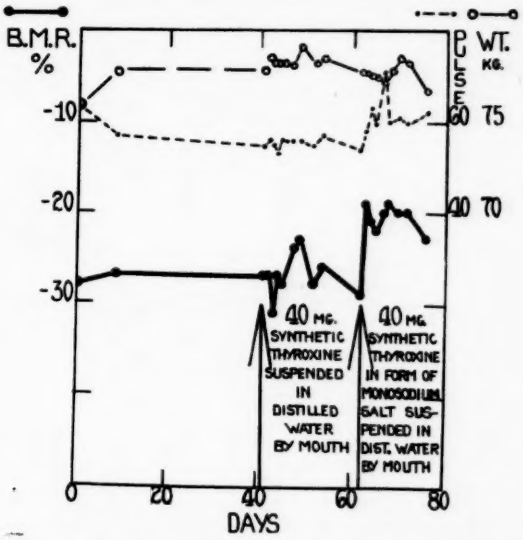
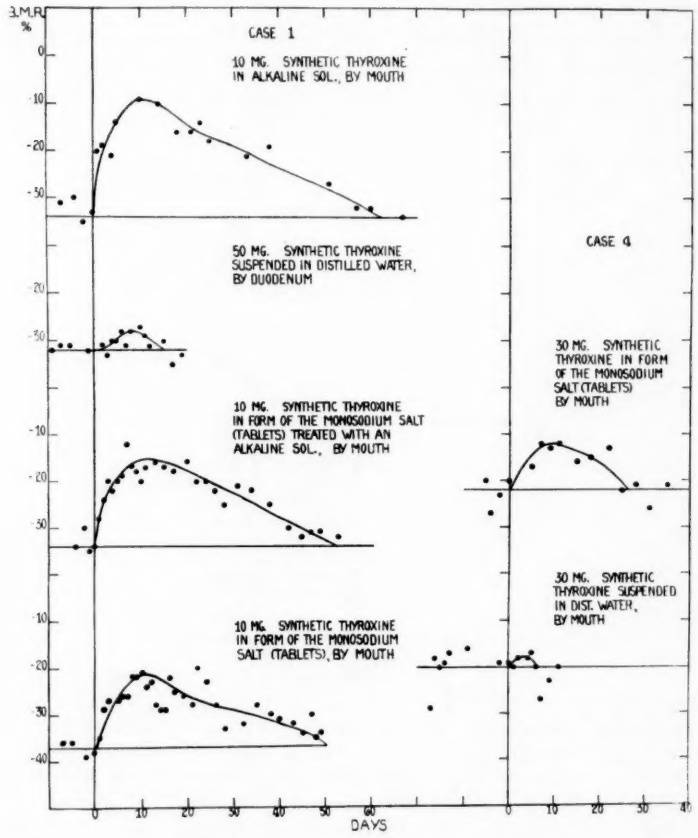


Chart 5 (Mrs. J. McH.; height, 163 cm.; age 42).—The oral dose of 40 mg. of pure synthetic thyroxine was administered at 4 p. m. on Jan. 31, 1933, in a suspension of distilled water, a total of 420 cc. being used, largely for rinsing. The patient had been fasting since the preceding night. The oral dose of 40 mg. of thyroxine in the form of its monosodium salt (41.2 mg. of the salt) was given at 1:25 p. m. on Feb. 21, 1933, in the form of tablets, which were washed down with 400 cc. of distilled water. The patient had been fasting since the preceding night.

routes are summarized in table 2, where, in order to facilitate comparison, they have arbitrarily been calculated in terms of the effect of 10 mg. of thyroxine by the method indicated in the footnotes. While the slight increase in basal metabolic rate caused by thyroxine as the free amino-acid is not regarded as definite, it may be noted that for every 10 mg. of thyroxine administered by mouth in the form of the monosodium salt the

basal metabolic rate increased 7 points (from minus 28 to minus 21 per cent) on the average; for every 10 mg. administered by mouth in akaline solution the basal metabolic rate increased 20 points (from minus 31 to minus 11 per cent) on the average, and for every 10 mg. injected intravenously in alkaline solution the rate increased 32 points (from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CASE 1
10 MG. SYNTHETIC THYROXINE IN ALKALINE SOL., BY MOUTH
50 MG. SYNTHETIC THYROXINE SUSPENDED IN DISTILLED WATER, BY DUODENUM
10 MG. SYNTHETIC THYROXINE IN FORM OF THE MONOSODIUM SALT (TABLETS) TREATED WITH AN ALKALINE SOL., BY MOUTH
10 MG. SYNTHETIC THYROXINE IN FORM OF THE MONOSODIUM SALT (TABLETS), BY MOUTH
CASE 4
30 MG. SYNTHETIC THYROXINE IN FORM OF THE MONOSODIUM SALT (TABLETS) BY MOUTH
30 MG. SYNTHETIC THYROXINE SUSPENDED IN DIST. WATER, BY MOUTH
DAYS

Chart 6.—Detailed data for computing the excess basal calories produced by administering thyroxine in various forms by mouth and duodenum to patients 2 and 4.

minus 37 to minus 5 per cent) on the average. Thus, thyroxine when administered by mouth in the form of the crystalline monosodium salt has only 35 per cent as much effect as when administered by mouth in alkaline solution and only 22 per cent as much as when it is administered intravenously in alkaline solution, while an alkaline solution is

only 63 per cent as effective when given by mouth as when given intravenously. These conclusions, of course, apply only to the administration of single large doses. The prolonged daily administration of small doses might produce somewhat different effects.

TABLE 2.—*Summary of Effects of Administering Thyroxine in Various Forms by Mouth and Duodenum Compared with Those of Administering It Intravenously in an Alkaline Solution*

Medication*	Iodine Content of Substance Administered, Mg.	Number of Patients	Number of Administrations	Average Basal Metabolic Rate Before Treatment, per Cent Normal	Average Level to Which Basal Metabolic Rate Rose, per Cent Normal	Average Change in Basal Metabolic Rate, Points	Average Change in Basal Metabolic Rate in Terms of Response to Intravenous Injection, per Cent†
10 mg. pure synthetic thyroxine suspended in distilled water, by duodenum	6.5	3	3	—30	—29	1	3
10 mg.‡ pure synthetic thyroxine suspended in distilled water, by mouth	6.5	4	4	—32	—30	2	6
10 mg.§ synthetic thyroxine in the form of its monosodium salt (tablets), by mouth	6.5	6	6	—28	—21	7	22
10 mg.‖ pure synthetic thyroxine in alkaline solution (sodium hydroxide), by mouth	6.5	5	5	—31	—11	20	63
10 mg.¶ pure thyroxine in alkaline solution, intravenously	6.5	6	8	—37	— 5	32	100

* The effect of a given dose of thyroxine was calculated in terms of 10 mg. by multiplying the number of points the basal metabolic rate changed following its administration by the fraction $\frac{10}{x}$, in which x = the dose of thyroxine used. All thyroxine referred to in this table was synthetic (Hoffmann-La Roche) except that so-called natural thyroxine (Squibb's) was used for four of the intravenous injections.

† For the calculation of the figures in this column the average change in the basal metabolic rate for each type of medication was divided by 32 and the result multiplied by 100.

‡ The patient not included in table 1 received a dose of 10 mg.

§ The two patients not included in table 1 received doses of 7.5 and 10 mg., respectively.

‖ All doses were 10 mg. except one of 7.5 mg. For the second patient of the present study we included for this summary of the effects of an alkaline solution by mouth only the effects of pure thyroxine dissolved in alkali, because of the uncertainty about all the monosodium salt being dissolved when the tablets of this substance were treated with alkali.

¶ All doses were 10 mg. except two of 7.5 mg. The thyroxine was dissolved in an excess of sodium hydroxide for all injections except one, for which it was dissolved in an excess of potassium hydroxide.

COMMENT

The fact that the oral or duodenal administrations of single large doses of from 10 to 100 mg. of pure thyroxine do not produce a measurable increase in basal metabolic rate is not proof that much larger doses would not have an effect. Data are now being collected on the first patient of this report which show that when a comparatively large amount of pure thyroxine is administered by mouth every day the basal metabolic rate may be held at the normal level. Final figures

are not yet available, but from data already collected, it would appear that the amount required for this purpose is about one hundred and fifty times as great as if the thyroxine were given intravenously in an alkaline solution. The great difference in the effects of the various forms of thyroxine by the oral route may be largely a matter of solubility, as previously suggested.[6]

Since thyroxine is soluble in alkali, it might be supposed that injecting it directly into the duodenum would result in some of it going into solution and hence being absorbed, because of the slight alkalinity of the duodenal contents. It would seem from our results, however, that this alkalinity is not great enough to result in the solution of more than a very small fraction of the amount administered.

SUMMARY

The oral administration of single doses of from 30 to 100 mg. of pure thyroxine suspended in distilled water is without a definite effect on the basal metabolic rate in patients with myxedema.

The duodenal administration of single doses of from 10 to 50 mg. of pure thyroxine suspended in distilled water is likewise without a definite effect.

From the data reported in this and two previous communications, it would seem that in patients with myxedema the oral administration of 10 mg. of thyroxine in the form of its crystalline monosodium salt increases the basal metabolic rate 7 points (from minus 28 to minus 21 per cent) on the average, and the oral administration of 10 mg. in alkaline solution increases the basal metabolic rate 20 points (from minus 31 to minus 11 per cent) on the average, while the intravenous administration of 10 mg. in alkaline solution increases the basal metabolic rate 32 points (from minus 37 to minus 5 per cent) on the average.

6. Footnotes 1 and 2a.

Book Reviews

Treatment in General Practice. By Harry Beckman, M.D., Professor of Pharmacology at Marquette University School of Medicine. Second edition, revised and entirely reset. Cloth. Price, $10 net. Pp. 889. Philadelphia: W. B. Saunders Company, 1934.

In looking over this compendium, the reviewer was impressed by its breezy style, its occasional personal opinion and its human quality. Having avoided books on therapy for many years, he was impressed by the amount of factual knowledge collected in these pages. Each disease is arranged in alphabetical order under subheads, which affords easy access to the matter in hand. The author has developed a distinct contribution to a book on treatment for quick reference. Having become interested in this style of presentation, the reviewer wandered into one of the most extensive county libraries in this country and asked the librarian about the former edition of the work. She stated that it is the most popular reference book on treatment on the shelves.

If any criticism might be made of this volume, it would be to advise the author to leave out all the tropical diseases which are not found in this part of the world. Adding references to the discussion would make the book too bulky. The names of authors are profuse enough in the text to permit more extensive reading.

The mass of data, observations, theories and polemics which make up medical literature is so vast-much being so inconsequential and much consisting of just words—that an effort of this kind, even though it must be revised as time goes on, is most commendable.

It is an excellent reference for general practice.

A Diabetic Manual for the Mutual Use of Doctor and Patient. By E. P. Joslin. Fifth edition. Price, $2. Pp. 224, with 50 illustrations. Philadelphia: Lea & Febiger, 1934.

In his usual lucid style Dr. Joslin has incorporated the essential features of the modern treatment of diabetes in a very readable book of two hundred and twenty-four pages. Although the book is designed primarily for instruction of the patient, it is equally useful to the physician who does not limit his practice to diseases of metabolism. Dr. Joslin's optimism and kindly interest in human beings is evident throughout. He understands diabetic patients as well as diabetes. He is fully aware of the many questions which arise to perplex the patient and answers these questions clearly and graphically. The book is illustrated liberally with photographs of diabetic patients enjoying life. Such photographs aid greatly in dispelling the horror of the disease so often encountered in dealing with patients who are beginning treatment.

The treatment of diabetes by diet is fully explained. Although the diets which the author recommends contain considerably more carbohydrate than those which he advocated in former years, none of his standard diets contain more than 172 Gm. of carbohydrate. This figure is considerably lower than that recommended by the advocates of the "high carbohydrate diet." Perhaps as the result of his experiences before the introduction of insulin, Dr. Joslin does not approve the use of diets low in carbohydrate and high in fat. He believes that these diets predispose to arteriosclerosis and the development of diabetic acidosis. In this respect his conclusions are at variance with those of some physicians in other clinics.

Numerous menus and recipes are given to exemplify the diets. A glance at the former is sufficient to indicate that the dietary life and consequently the social life of the diabetic patient today is much less ascetic than it was a few years ago. The work of the dietitian should be the expert and artistic application of the science of nutrition. The following menu, given for a lunch, illustrates how closely this ideal has been obtained: clear soup, steak, tomato and cucumber salad, peas, baked potato, butter, ice cream and saltines.

Since the introduction of insulin, diabetes has become a more chronic disease, and in consequence the incidence of degenerative complications appears to be

increasing. Considerable space in the manual is therefore given to the treatment of these complications and to their prevention by prophylactic hygiene. Careful study of these chapters, especially by middle-aged or elderly patients, would decrease greatly the needless occurrence of diabetic gangrene. The book is concluded with several interesting chapters on the heredity of diabetes, the relationship of diabetes to obesity, the treatment of diabetic children and the advisability of marriage of diabetic patients. The reviewer can recommend this book without qualifications.

Allergy in General Practice. By Samuel M. Feinberg, Assistant Professor of Medicine and Attending Physician in Asthma and Hay Fever Clinic, Northwestern University Medical School. Price, $4.50. Pp. 339, with 23 engravings and a colored plate. Philadelphia: Lea & Febiger, 1934.

In this book the author discusses the problems of allergy in a delightfully simple and straightforward manner. The book is evidently designed to stimulate readers interested in general medicine to realize that clinical allergy is frequently encountered, is recognizable and is often amenable to a fairly simple therapeutic program. It fulfils this purpose admirably.

The volume begins with an introductory chapter on the history of asthma and hay fever. There is next a short chapter dealing with the general principles of allergy and anaphylaxis, and then an excellent, more thorough discussion of asthma, with a consideration of its causes, diagnosis and treatment. The second half of the book deals with hay fever. O. C. Durham, chief botanist of the Abbott Laboratories, has contributed a chapter on the botanic aspects of hay fever, which will interest readers unfamiliar with such things as plants and pollens or the use of pollen surveys and weather maps in the treatment of hay fever. There is a short chapter on less clearly defined allergic conditions, such as eczema, angioneurotic edema, gastro-intestinal allergy and allergic headache. Finally, the book closes with a group of thirty-nine case reports to illustrate certain principles of diagnosis and treatment outlined in the text.

The entire book is written clearly and is very readable. Each chapter ends with a good bibliography. Medical students and general practictioners will find this volume an excellent one for reference and study.

News and Comment

INTERNATIONAL CONGRESS OF GASTRO-ENTEROLOGY

The First Congress of Gastro-Enterology will be held at Brussels, Belgium, Aug. 8 to 10, 1935. The secretary-general of the Congress is Dr. George Brohee, Rue de la Concorde, 64, Brussels. Dr. Max Einhorn, 20 East 63rd Street, New York, has been asked to form, and to act as chairman of, the North American Committee, and Dr. De Witt Stetten to serve as secretary of the committee.

CORRECTION

In the article by Dr. M. M. Wintrobe on "Anemia," in the August issue (ARCH. INT. MED. **54**:256, 1934), the fourth and fifth columns of section II in table 6 should read:

A. Sudden loss of blood	Acute posthemorrhagic anemia
B. Acute destruction of blood	e. g., hemolytic anemia caused by malaria

In other words, "e. g., hemolytic anemia caused by malaria" should appear on the third line opposite "B. Acute destruction."